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SOME EXPERIMENTS ON THE EXCHANGE OF FLUID BETWEEN THE BLOOD AND TISSUES.

By J. B. LEATHES, M.B. (*Oxon.*), F.R.C.S. (Two Figures in Text.)

(*From the Physiological Laboratory, Guy's Hospital.*)

THE experiments which form the subject of this communication were planned for the investigation of the movements of fluid passing to and from the blood vessels and the tissues. The observations group themselves under three heads.

(*a*) The changes in the volume of the blood that are effected by injecting into the veins of an animal hypertonic, isotonic, and hypotonic solutions of salt and sugar.

(*b*) The relation of the osmotic pressure of the lymph to that of the blood-serum, both under normal conditions, and under the abnormal conditions brought about by such injections.

(*c*) A comparison of the rates of lymph-flow induced by these means with the rates at which fluid leaves the blood vessels.

Method of Experiment.

Dogs anæsthetized with morphia and A.C.E. mixture were in all cases the animals employed. A tight ligature was secured round the renal vessels and ureter on each side, so as to exclude the kidneys from the circulation. A cannula was introduced into the jugular vein, and connected with a tube from which the solution to be injected was allowed to flow. A cannula in the femoral artery was connected with a manometer, and another on the other side was used for drawing off samples of the blood. In the cases in which the lymph was collected a cannula was placed in the thoracic duct close to its termination.

A sample of the blood was taken before the injection: the injection lasted from 4 to 13 minutes, and immediately it was finished another sample was taken, and subsequently at intervals further samples were taken till the end of the experiment. From the sample of

blood drawn before the injection 1 cubic centimeter was taken in a standardized pipette, and was mixed with 249 c.c. of water; and in the case of each of the later samples 1 c.c. was similarly measured and added to 99 c.c. of water. If the lymph was being collected, the samples of blood measured from 60 to 70 c.c. in order to secure enough serum to allow a determination of the freezing point with Beckmann's apparatus. Otherwise only two or three c.c. were withdrawn from the circulation for each sample.

The volume of the blood was estimated by hæmoglobinometry. For this purpose two tubes holding each about 15 c.c. and of exactly the same calibre were used: into one some of the diluted ($\frac{1}{250}$) normal blood was introduced, into the other 4 c.c. of each in succession of the diluted ($\frac{1}{100}$) samples that had been taken after the injection. To this second tube water was then added from a burette, till the tints of the hæmoglobin solutions in the two tubes were the same: the columns of fluid in the two tubes were made equal by adding when necessary more of the ($\frac{1}{250}$) standard normal solution to the first tube. The two tubes standing in a white porcelain stand, it was found that the range of error by this method of estimation was less than 2%. For the estimation of the freezing point of the lymph it was necessary to have 20 c.c., and so if the lymph was flowing slowly, a single sample of lymph might be obtained for two or three that were taken of the blood; but this was exceptional. The blood was centrifuged to obtain the freezing point of the serum; the lymph was not centrifuged, but the clot was broken up and the fluid poured off. As to the fluids injected, they were either isotonic with the blood, 1% NaCl solution or serum obtained from another dog, or hypertonic, a strong solution of dextrose or a 2% NaCl solution, or they were hypotonic, a ·3% solution of NaCl. In the case of the salt solutions and serum the same volume, 35 c.c. per kilog. of the body-weight or about 50% of the blood, was injected in every experiment: the sugar solutions contained 5 g. dextrose per kilog. of body-weight dissolved in a small volume of water, about 7 c.c. for every 5 gr.

A. *The changes in the volume of the blood.*

The facts obtained under this heading are most conveniently presented in the form of curves, some of which are given in Fig. 1 and Fig. 2.

The curves in Fig. 1 present the changes that were effected by injecting 5 g. dextrose per kilog. The ordinates represent the volume

of the blood, which is proportional to the dilution of the Hb.: the abscissæ represent the intervals of time between the different obser-

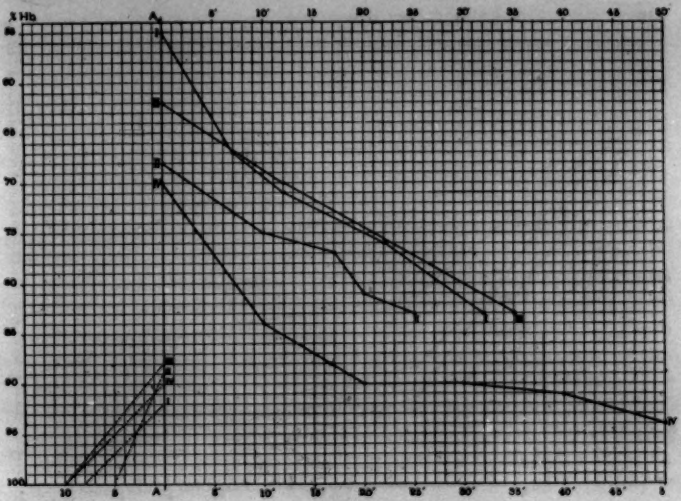

FIG. 1.

Injection of 5 g. dextrose per kg. body-weight.

Ordinates represent volume of blood as indicated by % of Hb.
Abscissæ represent intervals of time.
The line AA_1 marks the time at which the injection was finished in each experiment.
The dotted lines to the left of AA_1 indicate the theoretical dilution effected by the volume of fluid injected.

vations. The line AA_1 marks the moment at which the injection was ended and the first sample of blood was drawn for comparison with the normal blood, which was taken before the injection. To the left of the line AA_1 dotted lines indicate the duration of the injection, and also the change in the percentage of hæmoglobin effected by the addition to the blood of the amount of fluid injected: thus in the experiment quoted as No. I., Fig. 1, the amount of water used to dissolve the sugar was 6 c.c. for every 5 g., and therefore, if we suppose the original volume of the blood to have been 70 c.c. per kilog. of the dog's weight, would by itself have reduced the proportion of hæmoglobin to 92% of the original amount. The dotted line therefore represents

what may be called the theoretical dilution, and indicates how much of the dilution effected by the injection was due to the addition to the

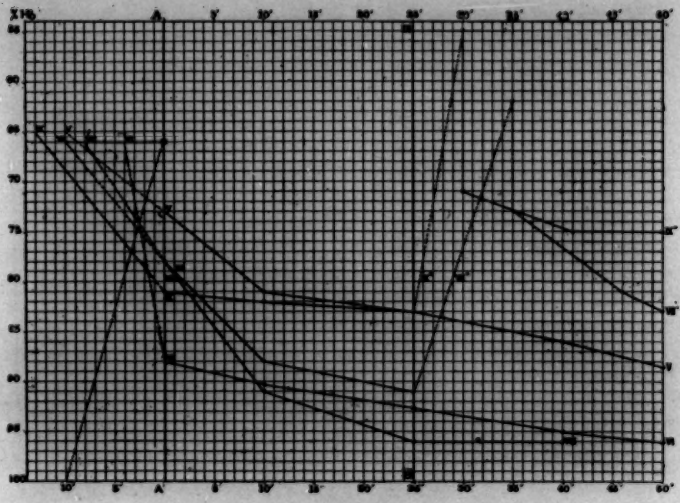

FIG. 2.

Injection of salt solutions and serum.

This figure is constructed as figure 1.

The line AA_1 marks the time at which the injection was finished.

The dotted lines to the left of AA_1 sloping down from left to right indicate the duration of the injection, and the proportion of the injected fluid which had left the vessels by the termination of the injection.

In each case the volume of fluid was 35 c.c. per kg. of the body-weight, giving theoretical dilution = 66 %. In Nos. V. and IX. the theoretical dilution was slightly more than this, about 65 %, owing to larger samples of blood having been taken.

The line BB_1 marks the time at which in Nos. VII. and IX. a second injection was given equal to the first in volume and in composition. The dotted lines to the right of BB_1 indicate the duration of these injections and the proportion of the fluid that had left the vessels by the end of the injection.

The solution in No. V. was 2 % NaCl.

" " Nos. VI. and VII. was 1 % NaCl.

" " No. VIII. was the serum from another dog.

" " No. IX. was ·8 % NaCl.

blood of the amount of fluid injected. In the experiments from which the curves in Fig. 2 were constructed, the same volume of fluid was

injected in each case, viz. 35 c.c. (in No. IX. 36 c.c.) per kilog. of the dog's weight, giving a theoretical dilution of 66%; and so the dotted lines representing the addition to the blood of this amount of fluid are not repeated for each case. But seeing that the actual dilution found at the end of the injection was in all these experiments less than the theoretical dilution, fluid must have left the vessels already by the time of the first examination of the blood. The amount of this fluid as well as the duration of the injection is indicated by the dotted lines sloping down from left to right to the different points on the line AA_1. In Nos. V. and IX. the theoretical dilution is estimated rather higher, viz. 65%, because the samples of blood taken before the injection were in these experiments larger, and had already effected an appreciable diminution in the actual amount of Hb. present in the body. In Nos. VII. and IX. a second injection like the first was made after 25 minutes had elapsed: the theoretical dilution effected by these second injections is indicated by dotted lines as before.

The chief facts of interest which are disclosed by a study of the curves in Fig. 1 are:

(1) The increase in the volume of the blood caused by the injection of 5 g. dextrose per kilog. is enormous, and quite out of proportion to that caused by the volume of the injection[1].

(2) This increase takes place with remarkable rapidity: in the experiment No. I. the volume of the blood was nearly doubled by the time the injection was finished. In eight minutes 100 c.c. had been injected and more than 800 c.c. had been taken up from the tissues. In fact the time necessary for this transfer of fluid is inappreciable; for the moment the process of raising the osmotic pressure of the blood was stopped, a sample of the blood serum had practically the same freezing point as the lymph which was collected during the two minutes following (vide Protocols at end of this paper); and therefore the transfer of fluid to the blood must also have stopped, seeing that the force which caused the transfer, osmosis, had ceased to act.

[1] This point has already been observed by Brasol in the *Archiv für Anatomie und Physiologie*, 1884: in a research on the fate of sugar introduced into the blood, he incidentally determined by hæmoglobinometry the change in the volume of the blood effected by injections of large quantities of sugar, and observed this enormous and rapid increase, and also the fact that in the course of two hours the volume gradually sank to normal again. Similar observations were made by Klikowicz for strong solutions of sodium sulphate (*Archiv für Anatomie und Physiologie*, 1886).

(3) From the moment at which the injection of sugar is stopped, the volume of the blood begins to diminish. As we have seen the restoration of osmotic equilibrium is already completed almost as soon as the last gramme of sugar is added to the blood, and the distended vessels then at once begin to unload themselves of their excess of fluid. The fall of the curve is at first rapid but it tends to become slower after a few minutes. In the experiment No. I. after allowance is made for the amount of blood that was removed for examination in the course of the experiment, the volume of the blood half-an-hour after the injection was within a few c.c. the same as it was before it[1].

Turning now to Fig. 2 it will be seen that in only one of the experiments (No. V.) was the fluid injected hypertonic. This is the only case therefore in which we could expect to find the volume of the blood at the end of the injection equal to or greater than that indicated by the theoretical dilution: as a matter of fact it was less. The forces tending to expel fluid from the vessels more than counterbalanced the osmotic force drawing fluid in from the tissues. In these experiments therefore we have only to compare the different rates of removal of the fluid from the vessels. The following points are noticeable:

(1) The isotonic solutions leave the vessels at different rates in different animals: thus in VI. and VII. the same volume of 1% NaCl solution was injected into two animals; in VI. the injection lasted 4 mins., in VII. 10 mins.; at the end of the injection in the former case much more fluid had left the vessels than in the latter. It may be tempting to explain this by supposing that rapid distention of the vessels enabled them to get rid of their excess of fluid more rapidly than a slower and more gradual distention. But against this is the fact that in No. VIII. the same volume of serum was injected at the slower rate, and at the end of the injection had been removed from the vessels to the same extent as the salt solution injected at the more rapid rate. Now if anything we should expect serum to leave the

[1] When comparatively large samples of blood had to be taken (in order to obtain the freezing point of the serum) the loss of hæmoglobin is of course not negligible and the volume of the blood is no longer inversely proportional to the percentage of hæmoglobin. Consequently to get the true curve for the changes in the volume of the blood, allowance has to be made for the quantity of blood drawn off as well as the proportion of hæmoglobin in each sample, and the curve so obtained approaches the normal more rapidly than the curves given in the figures. The removal of such small samples on the other hand as 2 or 3 c.c. at a time effects a reduction of the percentage of hæmoglobin and of the volume of the blood which is for present purposes negligible.

vessels more slowly than isotonic salt solution, since the mixture of the injected fluid and blood contains more albumen and should therefore filter more slowly. There can be little doubt therefore that different animals react differently to this test; though what the causes are which determine these differences we can only guess.

(2) The influence of osmotic differences on the curves in Fig. 2. That the isotonic fluids in Nos. VI. VII. and VIII. should leave the vessels more rapidly than the hypertonic solution in V. is only what would be expected. On injecting a quantity of 2 % NaCl solution we are injecting a fluid theoretically capable of drawing nearly an equal volume from the tissues; and this must of course retard the discharge of surplus fluid by the vessels. But when the fluid injected is strongly hypotonic, a force is introduced which must act in the opposite direction. The tonicity of the blood is lowered below that of the rest of the body, and every cell and tissue outside the vascular system and in the vessel-walls exerts a pull on the water in the vessels till osmotic equilibrium is re-established. But when we come to compare the curve given by ·3 % NaCl solution in No. IX. with the curves for isotonic injections in VI. VII. and VIII., it is obvious that in one of the latter No. VI. the fluid left the vessels much more rapidly than did the hypotonic solution in No. IX., and in the other two VII. and VIII. although by the end of the injection a smaller proportion of the fluid had been removed than at that epoch in No. IX., nevertheless the subsequent rate of removal is in these two experiments also very decidedly faster, so that after 25 mins. the vessels retained a smaller proportion of the isotonic injections than they did of the hypotonic. The same fact is shown by the curves for the second injections in Nos. VII. and IX., the hypotonic solution leaving the vessels at first more rapidly but afterwards more slowly than the isotonic. This is a curious fact and requires explanation. It is possible that hypotonic solutions exert some influence on the vasomotor system as Dr Starling has suggested to me, from observations of his on venous pressures: he found the venous pressure to be raised by hypotonic injections as by isotonic, but for a much shorter time. This observation may explain perhaps the fact that Cohnheim's plethora experiments, in which ·6 % NaCl was used, gave him a smaller rise of venous pressure than that found by Bayliss and Starling[1] working with 1 % NaCl solution. Bayliss[2] also has observed that if hypotonic salt solution is passed through the vessels of a limb at the same pressure

[1] This *Journal*, XVI. 183. 1894.

[2] Privately communicated.

as the original arterial pressure the limb swells. Perhaps therefore we may suppose that the effect of lowering the osmotic pressure of the blood is to increase the total capacity of the vessels, and for this reason the injected fluid in No. IX. was not expelled so rapidly as would have been expected.

The forces that are concerned in these changes in the volume of the blood are:

(1) *Osmosis:* this alone can be responsible for the great influx of fluid from the tissues to the blood in the experiments I.—IV. That it is therefore also responsible for the retardation of the removal of the hypertonic 2% NaCl solution in No. V. there can be no doubt. But in all these experiments, by the time the injection is finished, the osmotic pressure of the serum is no longer above that of the lymph, the inflow of fluid from the tissues has ceased, and the distended vessels proceed to rid themselves of their surplus fluid.

The only case in which the osmotic conditions can have helped in this process is in No. IX. where the osmotic pressure was lowered by the injection; and as in this experiment the osmotic pressure of the serum gradually rose during the 25 minutes for which it was observed after each injection, it is probable that osmosis was removing fluid from the vessels as well as bringing in salts. If so, however, seeing that the total amount of fluid removed from the circulation was very small, (only 35 c.c. in 25 mins. after the first injection and less than 100 after the second in the same time) there is not much scope left for the action of the other forces concerned in the discharging of the distended vessels[1].

(2) *Filtration:* when once osmotic equilibrium is established the curves for the experiments in which it had been raised closely resemble those of the experiments in which the injection was isotonic with the blood: the decline of the curve varies with the distention of the vessels. And that filtration was mainly concerned in the unloading of the distended vessels there can be no doubt.

(3) It is possible however that a third force comes into action here, the force recently suggested by Hamburger (in the *Archiv für Anatomie und Physiologie*, 1895, p. 348) in explanation of many of the phenomena of the transference of fluids in the animal body, which

[1] This is perhaps further evidence in favour of the suggestion made in a preceding paragraph, that hypotonic solutions paralyse the vessel-walls: for here the volume of the blood was much increased, and yet what little fluid left the vessels seems to have been removed by osmosis.

he calls molecular imbibition. The colloids of the blood are made by these injections to take up a much larger quantity of water than the colloids in the tissues. According to Hamburger's suggestion this will result in water being taken up by the less saturated tissue proteids from the more saturated proteids of the blood. At present so little is known of the action of any such force that it is impossible to say what it may be capable of, but the conditions for its action would seem to be present in these experiments.

B. *The Relation of the Osmotic Pressure of the serum to that of the lymph.*

(1) In all the experiments of which protocols are given here, as well as in all other cases in which I have taken the freezing points of the serum and normal lymph from the thoracic duct of dogs the freezing point of the lymph has been from ·005° to ·01° lower than that of the serum.

(2) The freezing point of the serum may be lowered or may be raised by the injection of saline solution according to its strength, but that of the lymph is always found to vary with it, and to bear almost exactly the same relation to it. On examining the figures given in the protocols it will be seen that whatever change is effected in the freezing point of the serum that of the lymph is always about ·01° lower: once it was ·02° lower but never more than that.

Now Hamburger has found larger differences than this[1]; and it is on this ground largely, though also because he finds the composition of the lymph varies in a way which does not correspond to the variations in the composition of the serum taken from the same animal at the same times, that he gives his support to the secretion theory of lymph-formation. How are these divergent observations to be reconciled? The lymph that Hamburger examined did not come from the thoracic duct, but from the cervical lymphatic of horses many of which at any rate were either eating or walking and drawing loads. Now the metabolism of muscle implies the breaking up of complicated molecules into less complicated, but more numerous molecules, and therefore must involve a rise of the osmotic pressure of the fluid in which the muscle fibres are bathed. And to assume as Hamburger does that the neck muscles of a horse that is walking, or still more working

[1] *Beiträge zur path. Anat. u. allgem. Pathologie*, XIV. 447.

against the collar, are at rest must surely be erroneous. Every movement of the fore-limbs and chest must alter the tension and activity of almost every muscle in the neck. Again when the horse is eating, not only are the muscles at work, but the salivary glands are secreting from the lymph a fluid having the osmotic pressure of a solution of only about ·5 % NaCl; and this too must raise the osmotic pressure of the lymph that is left to flow out of the cannula. So the fact that the cervical lymph under these conditions has a higher osmotic pressure than the serum may be fully accounted for by the metabolism of the tissues, and need not necessarily mean that it has been secreted by the blood capillaries against osmotic opposition.

C. *The ratio of the lymph-flow to the volume of fluid leaving the vessels.*

From the analysis which is given in tabular form at the end of the protocols with this paper, it will be observed that of the fluid which may be calculated to have left the vessels in a given time the fraction which appears as lymph flowing from the thoracic duct is very much smaller when the injection was hypotonic than in the other cases. The reason for this is sufficiently obvious. In experiments I.—IV. the fluid that left the vessels was isotonic with the lymph, and probably with the fluid in the tissues and tissue-cells, which had given up so much water to the blood during the time that the osmotic pressure of the blood was being raised by the injected sugar. The fluid that left the vessels in these cases was in the main at any rate being driven out of the distended vessels by filtration, and its destination was simply any space where it would be under less pressure than in the vessels. In exp. IX. the fluid was drawn from the blood by the higher tonicity of the tissues and was detained by the tissues to lower their osmotic pressure till it was equal to that of the blood: a comparatively small portion of it therefore was left free to fill the lymphatic spaces and flow out by the thoracic duct. Dr Starling tells me that again and again he has observed that the increase of lymph-flow caused by hypotonic salt solutions injected into the blood is much less than that caused by injections of the same amount of normal saline.

It is clear from this that the lymph-flow does not depend solely on the volume of fluid thrown off by the blood vessels. It emphasizes the necessity of remembering always that the quantity as well as the

composition of lymph flowing from lymphatic vessels depends as much on the action of the tissues as on that of the vessels.

A brief summary of the main points indicated by these experiments may be given in conclusion:

(1) Changes in the osmotic pressure of the blood are compensated with extreme rapidity by the transfer of fluid from tissues to blood, or from blood to tissues, when the kidneys are excluded from the circulation.

(2) There is no evidence that the vessel-walls play other than the part of a passive membrane in this interchange of fluids. They cannot be said to have the power of actively regulating the composition of the circulating blood.

(3) The osmotic pressure of the lymph from the thoracic duct is always slightly above that of the blood. This slight difference is not affected by alterations in the osmotic pressure of the blood, and is more easily accounted for by metabolism in the tissues, than by any active function of the vessel walls.

October 28, 1895.

PROTOCOLS.

No. 1. 28.6.95. *Injection of 5 g. dextrose per kg. body-weight.* ♂ dog weighing 17 kg. Kidneys ligatured and cannulæ as described in text.

		Δ		Δ	Hb
11° 30′—12° 0′	Lymph = 20 c.c. (with pressure on abdomen)	−·610			
12.10			Bled 60 c.c.	−·605	100 %
12.10–18	Injection of 85 g. dextrose in 100 c.c. water at 40° C. = 5 g. in 6 c.c. per kg.: theoretical dilution of Hb = 92 %.				
12.10–18	Lymph = about 20 c.c.				
12.19			Bled 60 c.c.	−·695	55 %
12.18–20	Lymph = 20 c.c.	−·705			
12.20–25	„ = 41 c.c.				
12.25			„ 60 c.c.	−·695	67 %
12.25–27	„ = 20 c.c.	−·705			
12.27–30	„ = 28 c.c.				
12·30			„ 60 c.c.	−·690	71 %
12.30–33	„ = 20 c.c.	−·698			
12.34–40	„ = 30 c.c.				
12.40			„ 60 c.c.	−·695	76 %
12.40–46	„ = 20 c.c.	−·700			
12.46–50	„ = 12 c.c.				
12.50			„ 60 c.c.	−·695	83 %

No. 2. 26.6.95. *Injection of 5 g. dextrose per kg. body-weight.* ♀ Dog weighing 5 kg. Kidneys ligatured and cannulæ as before.

			Hb
3° 36′—4° 57′	Lymph = 2·6 c.c.		
4.48		Bled 5 c.c.	100 %
4.57—5.2	Injection of 25 g. dextrose in 40 c.c. water at 40° C. = 5 g. in 8 c.c. per kg., giving theoretical dilution of Hb = 90 %.		
5.2		Bled 3 c.c.	68 %
4.57—5.2	Lymph = 10 c.c. (with some obstruction in cannula)		
5.10–12	Lymph = 10 c.c.		
5.12		„ 2·5 c.c.	75 %
5.12–17	„ = 12 c.c.		
5.19		„ 3 c.c.	77 %
5.22		„ 5 c.c.	81 %
5.17–23	„ = 13 c.c.		
5.27		„ 5 c.c.	83 %
5.23–3	„ = 10 c.c.		

Lymph came too slowly in this small dog to obtain enough before the injection for the estimation of the freezing point.

No. 3. 17.6.95. *Injection of 5 g. dextrose per kg. body-weight.* Dog weighing 9 kg. Kidneys ligatured and cannulæ as before.

		Δ		Δ	Hb
12° 20′—1° 40′	Lymph = 25 c.c.	−·620			
1.40—1.50	„ = 3 c.c.				
2.0			Bled 80 c.c.	−·610	100 %
2.40–50	Injection of 45 g. dextrose dissolved in 75 c.c. water at 40° C. giving theoretical dilution of Hb = 88 %.				
2.50			Bled 80 c.c.	−·725	62 %
2.50—3.20	Lymph = 25 c.c.	−·720			
3.2			„ 60 c.c.	−·715	70 %
3.25			„ 50 c.c.	−·685	83 %

Lymph during the injection (2.40–50) not measured.

No. 5. 7.8.95. *Injection of 35 c.c. 2 % NaCl solution per kg. body-weight.* ♂ collie, weighing 28·5 kg. Kidneys ligatured and cannulæ as before. The thoracic duct was small, and the lymph flow before the injection was very slow, not more than 1 to 2 c.c. in 10′, the cannula being constantly blocked with clot.

		Δ		Δ	Hb
1° 45′			Bled 75 c.c.	−·600	100 %
1.52—2.2	Injection of 1000 c.c. 2 % NaCl solution at 40° C. = 35 c.c. per kg. giving theoretical dilution of Hb = 65 %.				
1.52—2.2	Lymph = 20 c.c.				
2.2			Bled 60 c.c.	−·675	73 %
2.2–4	„ = 20 c.c.	−·680			
2.4–12	„ = 34 c.c.				
2.12			„ 65 c.c.	−·655	81 %
2.12–17	„ = 20 c.c.	−·665			
2.17–27	„ = 30 c.c.				
2.27			„ 60 c.c.	−·645	83 %
2.28–35	„ = 20 c.c.	−·660			
2.35–42	„ = 12 c.c.				
2.42			„ 60 c.c.	−·640	86 %
2.42–58	„ = 20 c.c.	−·660			
3.0			„ 60 c.c.	−·640	90 %

No. 9. 29.7.95. *Injection of* 36 *c.c.* ·3 % *NaCl per kg. body-weight.* ♂ dog weighing 20 kg. Kidneys ligatured and cannulæ as before.

		Δ		Δ	Hb
12° 52′—1° 0′	Lymph = 10 c.c.	−·630			
2.0—2.10	„ = 5 c.c.				
2.15			Bled 70 c.c.	−·625	100 %
2.20–33	Injection of 720 c.c. ·3 % NaCl at 40° C. = 35 c.c. per kg. giving theoretical dilution of Hb = 65 %.				
2.20–33	Lymph = 5 c.c.				
2.33			Bled 70 c.c.	−·590	81 %
2.33–58	Lymph = 20 c.c.	−·615			
2.43			„ 65 c.c.	−·590	82 %
2.58			„ 65 c.c.	−·612	83 %
3.3–8	Second injection of 720 c.c. ·3 % NaCl giving theoretical dilution of Hb = 60 %.				
3.8			Bled 64 c.c.	−·545	70 %
3.18			„ 75 c.c.	−·550	75 %
3.8–20	Lymph = about 30 c.c. (some being lost at 3.10 owing to movements of the dog, which also increased the rate of flow)	−·560			
3.33			„ 75 c.c.	−·575	75 %
3.20—4.8	Lymph = about 30 c.c.				

Analysis of Protocols.

Throughout this paper in all calculations the normal volume of the blood is taken at 70 c.c. per kilogram of the dog's weight.

No. of Experiment	I	II	III	V	IX	IX″
Weight of dog	17 k.	5 k.	9 k.	28·5 k.	20 k.	
Fluid taken up from tissues by the blood during the injection	824 c.c. in 8′	124 c.c. in 5′	262 c.c. in 10′	—	—	—
Fluid given up to the tissues by the blood (i) during the injection,	—	—	—	290 c.c. in 10′	408 c.c. in 13′	471 c.c. in 5′
(ii) after the injection	644 c.c. in 32′	93 c.c. in 25′	193 c.c. in 35′	475 c.c. in 58′	35 c.c. in 25′	96 c.c. in 25′
Rate of lymph-flow before the injection	6·6 c.c. in 10′	·3 c.c. in 10′	3 c.c. in 10′	2 c.c. in 10′	5 c.c. in 10′	—
Rate of lymph-flow during the injection	20 c.c. in 8′	10 c.c. in 5′	not measured	20 c.c. in 10′	5 c.c. in 13′	—
Rate of lymph-flow immediately after the injection	80 c.c. in 10′	31 c.c. in 10′	8 c.c. in 10′	54 c.c. in 10′	8 c.c. in 10′	25 c.c. in 10′
Total amount of lymph given up by the thoracic duct from the beginning of the injection to the end of the experiment	211 c.c. in 40′	55 c.c. in 36′	—	176 c.c. in 68′	about 65 c.c. in 73′	—
Ratio borne by the total amount of lymph from thoracic duct to total amount of fluid leaving the vessels	$\frac{211}{644}$ in 40′ = ·3 (nearly)	about $\frac{40}{93}$ in 25′ = ·43	—	$\frac{176}{782}$ = ·21	$\frac{65}{1010}$ = ·06	—

ON THE ASSERTED EFFECT OF LIGATURE OF THE PORTAL LYMPHATICS ON THE RESULTS OF INTRAVASCULAR INJECTION OF PEPTONE. By ERNEST H. STARLING.

(*From the Physiological Laboratory, Guy's Hospital.*)

In a recent paper[1] Professor Gley describes experiments to show that a normal condition of the liver cells is essential to the ordinary effect of peptone in preventing coagulation of the blood.

In these experiments he ligatured the portal lymphatics, and found that a subsequent injection of peptone was without effect on the blood. Ligature of the bile ducts also interfered with the anti-coagulative action of peptone, but did not quite annul it.

I was extremely surprised with the results of M. Gley's experiments since in earlier experiments of my own, in which I had ligatured the portal lymphatics, injection of peptone had the usual effect—viz.: an abolition of the coagulability of the blood.

I have therefore repeated these experiments, and am quite unable to confirm M. Gley's results. I find that ligature of the portal lymphatics and bile ducts is absolutely without effect on the results of intravascular injection of peptone.

I give here short protocols of three of these experiments. In each one the lymph flow was observed; and the success of the ligature may be estimated from the very small results of the peptone injection on the lymph flow. (As I have shown previously, the effect of the first class of lymphagogues on the lymph flow can be almost or entirely abolished by shutting off the liver lymph from the thoracic duct.)

Exp. I. Dog 10 kilos. (June 14, 1893.)

Time.	Lymph.
3.10–20	= 5 c.cm.
3.20–30	= 5 c.cm. (From 20 to 35 ligatured portal lymphatics.)

[1] MM. E. Gley et V. Pachon. *Archives de Physiologie*, October, 1895, p. 711.

Time.	Lymph.	
3.50—4.0	= 3·2 c.cm.	(From 4.0 to 4.3 injected 5·5 grams peptone.)
4.0–10	= 6·2 c.cm.	
4.10–20	= 5·6 c.cm.	
4.20–30	= 4·5 c.cm.	

Dog killed at 4.55 by bleeding. The blood so obtained was still fluid on the following day.

Exp. II. Dog 6 kilos. (Oct. 23, 1895.) Portal lymphatics ligatured at 2 p.m.

Time.	Lymph.	Blood.
2.20–30	= 1·8 c.cm.	
30–40	= 1 c.cm.	(Blood sample at 2.34. Solidly clotted at 2.45.)
40–50	= 1·4 c.cm.	

From 2.50 to 52 injected 3 grams of peptone. After 2 or 3 minutes a fall of blood-pressure and dyspnœa.

2.52. (Blood sample fluid the following day.)

55—3.5	= 6·4 c.cm.	(At 2.55 blood sample taken. Fluid next day.)
3.5–15	= 3·5 c.cm.	(At 3.10 blood sample. Fluid next day.)
15–25	= 2·8 c.cm.	
25–30	= 1·4 c.cm.	(Dog killed at 3.30 by bleeding. The blood thus obtained still fluid on following day.)

Exp. III. Dog 9 kilos. (Oct. 25, 1895.) Portal lymphatics ligatured.

Time.	Lymph.	
3.50—4.0	= 5·4 c.cm.	(Blood sample at 4.6. Firmly clotted at 4.10.)
4.0–4.10	= 4.2 c.cm.	(4.11 to 4.13 injected 4·5 grams peptone.)
4.15–25	= 9 c.cm.	(Blood sample at 4.14.)
25–35	= 4·2 c.cm.	(Blood sample at 4.25.)
35–45	= 4·0 c.cm.	(Blood sample at 4.35.)

4.50. Dog killed by bleeding. The next day this blood, and all the samples of blood taken subsequent to the injection, were still fluid.

How are we to explain this divergence of experimental results?

In an earlier paper[1], I described four experiments in which peptone was injected after ligature of the kidney vessels on both sides. In 3 out of the 4 experiments the coagulability of the blood was not affected by the peptone. I pointed out at the time however that this number of experiments was not sufficient "to decide whether this deviating result is to be referred to the operation, or to an accidental agglomeration of immune dogs."

[1] This *Journal*, xiv. 150. 1893.

Since then I have repeated these experiments several times, but have never again obtained the same results. Ligature of the renal vessels does not alter the normal effect of peptone injection.

I have thought that possibly the common feature of my earlier kidney experiments and the experiments of M. Gley, might consist in a certain degree of shock, due to exposure of intestines, or injury of splanchnics. Several experiments which I have made however lend no support to this view. Exposure of all the intestines for an hour, ligature of the intestines in several places, with division of the splanchnic nerves, crushing the suprarenals—all these procedures had no influence on the effects of a subsequent peptone injection. In all cases the blood was rendered uncoagulable by the peptone.

On the other hand everyone has met with apparently perfectly healthy dogs in whom a peptone injection had no influence on the coagulability of the blood, and I am therefore inclined to explain M. Gley's results in the same manner as I explained those of my kidney experiments, viz. :—to an accidental aggregation of immune dogs.

THE RESPIRATORY EXCHANGE OF THE LOWER MARINE INVERTEBRATES.

By H. M. VERNON, B.A.

(Thirteen Figures in Text.)

(*From the Zoological Station, Naples.*)

THE subject of the respiratory exchange of the lower marine animals has an interest quite apart from the mere measurement of the exchange of material taking place in particular individual animals, because of the comparatively slight differentiation the tissues have in many of them undergone. In the higher animals the respiratory exchange is the sum of the changes taking place in various tissues, which differ considerably from each other both in their structure and chemical nature. Also the respiration is as a rule largely influenced and governed by stimulation and inhibition from nervous centres. In animals such as *Beroe* and *Cestus*, however, the nervous system is exceedingly primitive, so that we are probably justified in looking upon the respiratory exchange as almost entirely a pure tissue metabolism phenomenon, uncomplicated by nervous influence. Now in the transparent pelagic animals such as Medusæ, Ctenophora and Salpæ, there appears to external observation but slight differentiation of the viscid protoplasmic tissue, and so it seemed of interest to determine whether, within the limits of experimental error, unit weight of such animals has at the same temperature the same respiratory exchange, and whether the relation of the respiratory activity to temperature is in the different animals constant.

The respiratory exchange of marine animals has hitherto been studied almost exclusively by Jolyet and Regnard[1]. Their method of experiment is a modification of that used by Regnault and Reiset for measuring the respiration of air breathing animals. As it necessitates the constant bubbling of a stream of air through the water in which the animal under examination is placed, it is quite impossible to use it for measurements of the respiration of delicate pelagic animals, and so another method had to be devised. The method adopted is

[1] *Archives de Physiologie*, 2 Sér. 4. 44, 584.

theoretically a very simple one. It consists in confining the animal in a closed chamber of water for a sufficient time, and then drawing off the water, boiling off the gases, and analysing them. The gases in a specimen of the same water having previously been determined, the increase in the carbonic acid, and the decrease in the oxygen, due to the respiration of the animal, are found. The practical application of this method proved to be by no means easy, the accurate determination of the carbonic acid evolved being especially difficult.

The Apparatus employed.

The apparatus finally adopted was arranged as is shown in the figure. The respiration chamber consisted of a small bell-jar with two

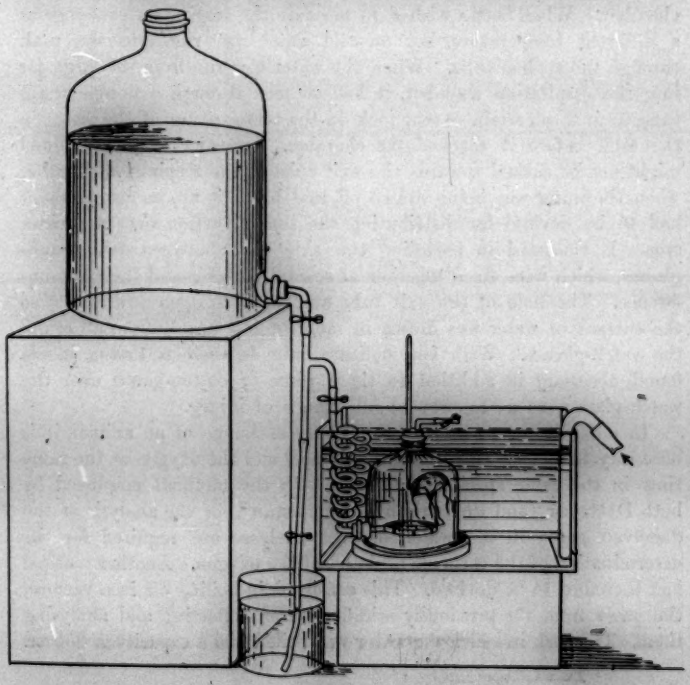

FIG. 1.

tubulures. Through the upper one passed the thermometer, and through the lower the tube by which the water was introduced and withdrawn (fig. 1). The bell-jar was provided with a wide pflange, so that its connection with the greased ground glass plate could easily be made quite tight. By means of the T tube, water could either be run from the large jar into the animal chamber, or could be drawn off into another vessel for analysis, or the water could be drawn off from the animal chamber for analysis. The large jar had a capacity of 16·5 litres, whilst the respiration chamber generally used held 761 grams of water. With larger animals it was sometimes replaced by one holding 1430 grams. The glass tank in which the respiration chamber was placed held about ten litres of water. It was always filled with salt water, and not with fresh, in case there should be any leak in the chamber. When it was wished to measure the respiratory exchange at a different temperature, hot or cold water was run into the tank through the syphon tube. When the water was run from the large jar into the respiration chamber, it had to pass through a double worm tube, so it to a certain extent took up the temperature of the water in the tank before it reached the chamber. In order that the animal might not be sucked towards the exit tube of the respiration chamber when the water was being drawn off, and block it up, an arrangement had to be devised for distributing the suction action over a greater area. It consisted in enclosing the exit tube between two watch-glasses, which were fixed together at several places round their circumference. The hole of the exit tube was made to open downwards, so the current of water was drawn in more or less evenly from all round the watch-glasses. With very delicate animals, such as *Cestus*, it was found necessary in addition to tie a piece of cotton-gauze over the watch-glasses, so as to prevent all chance of injury.

In order to determine the respiratory exchange of an animal, it is necessary to analyse both the carbonic acid and the oxygen at the same time in the same specimen of water. In the methods employed by both Dittmar[1] and Petterson and Eckman[2], for the analysis of the dissolved gases in sea water, distinct analyses are required for the determination of the carbonic acid and of the oxygen. Another method had therefore to be devised. This consisted in boiling off in a vacuum the gases from the previously acidified water, collecting and analysing them. The flask in which the water was boiled had a capacity of 350 c.c.

[1] *The Challenger Reports*, I.

[2] *Kongl. Svenska Vetenskaps Akademiens Handlingar*, XXIV.

This flask was connected by means of an inverted condenser with a Pflüger's mercury pump, as shown in the figure.

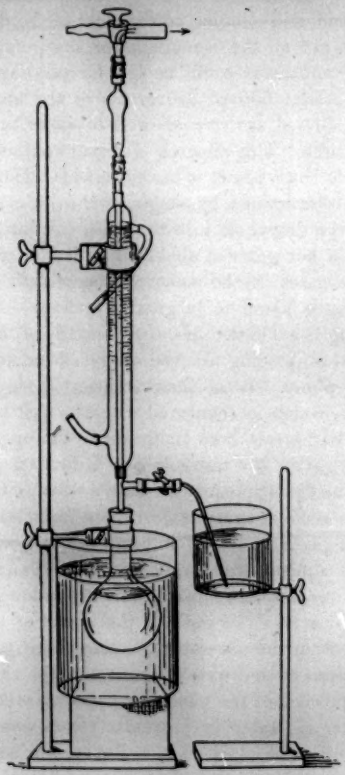

FIG. 2.

In performing an experiment, 10 c.c. of dilute sulphuric acid (containing 1 c.c. of the concentrated acid) were run into the flask, which was then connected with the condenser by means of a well greased caoutchouc cork. The greater part of the air was now pumped off, but in order to remove all the air from the apparatus, it was found to be necessary to warm the flask by placing a jar of hot water under it.

After the dilute sulphuric acid has boiled a few minutes, every trace of air can be pumped off. If the flask is not warmed in this manner, as much as two cubic centimetres of air remain behind, dissolved in the dilute sulphuric acid, and clinging to the sides of the flask. Very great care was taken to get all the connections of the pump and flask quite tight, so that the apparatus could be left for two days without a trace of air entering. About 300 to 330 grams of the water of which the gases are to be analysed are now allowed to enter the evacuated flask through the side tube. This amount of water contains about 30 c.c. of gas, of which more than 24 c.c. is carbonic acid. The exact amount of water analysed is determined by weighing the jar containing it before and after it has been drawn off into the flask. A jar containing about a litre and a half of hot water at about 36° C. is now placed underneath the flask, and the gases in the water are boiled off. This method of heating the water was found to be greatly preferable to a gas flame, as all violent bumping is avoided. About five-sixths of the gas boils off in the first five minutes, it being allowed to pass over into the large bulb of the Pflüger pump. It is then transferred directly to the gas analysis apparatus, which is connected with the exit tube of the pump by a short piece of narrow bore indiarubber tubing. The remaining fifth or so of the gas in the water is now boiled off, and then driven over into the gas analysis apparatus. Half-an-hour's boiling was found to be sufficient to remove every trace of gas from the water. As the last portion of the gas is boiled off in practically a perfect vacuum, less the tension of the aqueous vapour, it comes off comparatively readily. In this boiling off process care must be taken to allow as little water as possible to distil over into the bulb of the pump, as some of the carbonic acid evolved from the sea water becomes thereby reabsorbed. By having a quick stream of cold water passing through the condenser, and by only opening the cock of the pump for a second every now and then, all but about ·2 c.c. of water is prevented from passing over. The gases thus boiled off were analysed in a Petterson's apparatus. This was found to be very convenient and readily workable, and also exceedingly accurate for the purpose. As can be seen from the table in Appendix A, the average error in the analyses of the oxygen in the water is only ·09 c.c. per kilogram of water, or, as about 300 grams of water were analysed at a time, the actual error is only ·03 c.c. Because of the absorption of carbonic acid by the varying traces of water distilling over into the pump, the error is in this case larger. On an average it is ·27 c.c. per kilogram, or about ·08 c.c. in the weight of water actually

analysed. As the water contained about 70 c.c. of carbonic acid per kilogram, this is an error of only ·3 % in the determination. The operations of boiling off the gases and analysing them could be completed in from an hour to an hour and a half.

In the performance of an experiment on the respiratory exchange of an animal, the following method of procedure was adopted. The bell-jar or animal chamber was inverted, and the watch-glass contrivance being removed, the animal was introduced. The watch-glass contrivance being replaced, and the ground glass plate pressed firmly on, the bell-jar was reinverted, and placed in the tank of water. As much of the water as possible was now run off from the bell-jar, and fresh sea water from the large jar run in in its place. After a few minutes this water was run off, and more introduced. This process was repeated once again before the water, which was to be subsequently analysed, was run in. This second washing out of the contents of the bell-jar was necessary, as it was impossible to draw off all the water from the chamber. Some 70 c.c. remained behind, besides that contained in the animal itself, so that even after the double washing out, some ·2 to 1 % of the original water in which the animal was kept must have remained behind. Of course the composition of the gases in this water may have been very nearly the same as that of the gases in the water in the large jar, but as this was not known for certain, the precaution of a double washing out had to be taken. The time was noted at which the water in which the animal was to be left respiring was run in, and also the temperatures of the water in the bell-jar and in the tank. The temperature of the water was noted from time to time, and a mean of the different numbers taken as the temperature of the experiment. After the animal had been left in the closed chamber for a sufficient time, generally two or three hours, the water was allowed to run off from the chamber into a jar. The first 40 c.c. was rejected, as it had been contained in the worm and T tube, and had not entered the animal chamber at all. After as much as possible of the water had run off, it was stirred round gently once or twice, and the jar containing it weighed as rapidly as possible. About 300 grams of it was now, as described above, run into the evacuated flask for analysis. About three minutes was taken for the water to run off from the animal chamber, and a minute for the weighing. During this time the water was exposed to the air, and was consequently losing some of its carbonic acid, and absorbing oxygen. Control experiments, the details of which are given in Appendix A, showed that the change in the composition of the gases during this short time has

practically no influence on the results. The bell-jar was now washed out with more fresh sea water, as before, and a fresh determination of the respiratory exchange, generally at some other temperature, begun. In this determination, it is known that approximately 2 % of the water of the previous determination remained in the bell-jar, and as the composition of the gases in this water is known, it was possible to apply a correction for it. When the larger bell-jar was used it was determined that a correction for only 1 % of the water previously present had to be applied.

The large jar of water was filled fresh every day with water from the large tank of the Aquarium, which held several thousand gallons, as the gases in this water were subject to slighter variations in composition than the gases in water obtained from smaller tanks. It was nevertheless necessary to analyse the gases in the water every day, as the diurnal variations in their composition, though small, were irregular and not to be foreseen. In fact, the water was frequently analysed twice each day and the mean taken, in order that the experimental error in the determinations might be reduced as much as possible.

Having thus determined the composition of the gases in the fresh sea water, and also in some of the same water in which the animal had been respiring a known time, it is easy to determine the respiratory exchange which has taken place. Thus the total weight of water contained in the bell-jar is known, and so, the gases in a known portion of it having been analysed, the actual amounts of carbonic acid excreted and oxygen absorbed by the animal can be calculated. In these calculations no allowance was made for the water displaced by the animal in the bell-jar. It was assumed that the gases in the water in its tissues underwent a change of composition similar to that in the rest of the water in the jar.

The Results obtained.

The number of pelagic animals available for determinations of the respiratory exchange is comparatively small, as they have to be obtainable in fairly large quantities, and also to be a certain size. Altogether sixteen animals were experimented on, but anything like complete sets of observations were only obtained in the case of twelve of them. The animals examined consist of one species of the Protozoa, six of the Cœlenterata, four of the Mollusca, three of the Chordata, and two of the Vertebrata. Observations on various fish could of course have been multiplied indefinitely if desired, but it was thought that determinations

on two species would be sufficient for comparison with the results obtained for the lower animals. In the table are given the names of the animals experimented on, those on which only very few observations were made being marked with an asterisk.

PROTOZOA.	Radiolaria.	*Collozoum inerme**.
CŒLENTERATA	Hydrozoa.	*Carmarina hastata.*
		*Ægineta flavescens**.
		*Forskalia contorta**.
	Scyphozoa.	*Rhizostoma pulmo.*
	Ctenophora.	*Beroe ovata.*
		Cestus veneris.
MOLLUSCA	Gasteropoda.	*Tethys laporina.*
	Heteropoda.	*Pterotrachea coronata.*
	Pteropoda.	*Cymbulia peronii**.
	Cephalopoda.	*Octopus vulgaris.*
CHORDATA	Tunicata	*Salpa pinnata.*
		Salpa tilesii.
	Acraniata.	*Amphioxus lanceolatus.*
VERTEBRATA.	Pisces.	*Heliases chromis.*
		Serranus scriba.

Observations on Cœlenterata.

The observations made on the Cœlenterata will be discussed first, as these animals were studied at greater length than those belonging to the other groups. Of the Medusæ, complete series of observations were made on *Carmarina* and *Rhizostoma*, twenty-three determinations under normal conditions being made in the one case, and twenty-seven in the other. These animals are very favourable for the measurement of the respiratory exchange, as they can be kept longer in captivity than most pelagic animals, some of them living several weeks. In Figs. 3 and 4 are shown diagrammatically the values obtained for the respiratory activity at different temperatures. These values represent the amounts of oxygen absorbed, and not the carbonic acid excreted, as the oxygen values were found to be much less affected by experimental error. The ordinates represent the temperature of the experiment in degrees Centigrade, and the abscissæ the oxygen absorbed per kilogram body weight of the animal per hour, in decimilligrams. The respiration was only measured between the temperatures of 10° C. and 24° C., as on the

one hand it is very difficult with the apparatus employed to maintain the water at a uniform temperature much below 10° C., and on the other

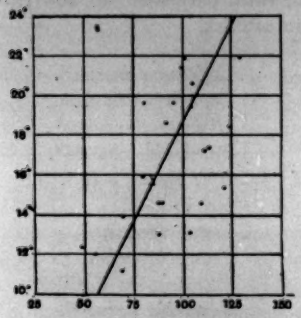

FIG. 3. Camarina hastata.

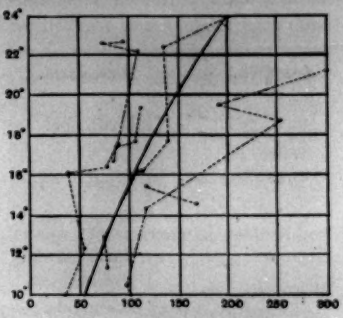

FIG. 4. Rhizostoma pulmo.

hand at temperatures much above 24° C. the animals are paralysed and killed by the heat. In the figures are drawn the curves which agree best with all the different determinations, and which therefore represent the relation of the mean respiratory activity to temperature. In the case of *Carmarina*, this curve is in best agreement with the different values when it is represented by a straight line. That is to say, the relation of the respiratory exchange to temperature appears to be constant. In the case of *Rhizostoma*, the values are better represented by a curved line. The values for *Carmarina* are fairly evenly distributed about the mean curve, but some of them diverge considerably from it. This is in very great degree due to different individual animals of different size and in different condition being used in the various experiments. Eight distinct series of experiments were made, in three of which only one medusa was used, and in the other five two or more medusæ. In the case of *Rhizostoma*, the values exhibit a much greater divergence from the mean curve. This is evidently due in very large part to distinct differences in the respiratory activity of different individual medusæ. Thus the values obtained for the same Medusa at different temperatures have been joined in the figure by dotted lines. It will be seen that the curves for each animal are almost entirely distinct, though the respiratory activity is affected to more or less the same extent in each case by temperature. One of the chief factors which determines the respiratory activity of an animal appears to be its size. Thus the dotted curve to the extreme left of the diagram is for a

medusa weighing 107 grams, whilst that to the extreme right is for one weighing 62 grams. The intermediate curves are for Medusæ of intermediate weight. This question of the influence of size on the respiratory activity will be referred to subsequently in the cases of the other animals examined.

In Figs. 5 and 6 are shown the values obtained for the Ctenophores *Cestus* and *Beroe*, seventeen determinations under normal conditions being made in the one case, and twenty-three in the other. With one

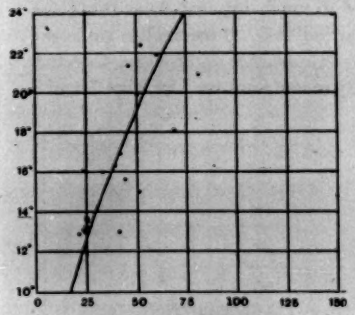

FIG. 5. Cestus veneris.

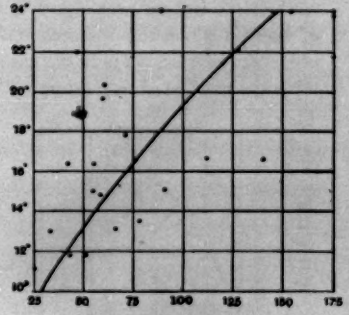

FIG. 6. Beroe ovata.

exception, the *Cestus* values are in very good agreement with the mean curve. This is the more satisfactory, as *Cestus* is a very difficult animal to work with. It is so delicate that the least rough treatment causes it to break in pieces and disintegrate. In washing out the animal chamber between two experiments, not so much of the water could be withdrawn as with less delicate animals, which can be drained almost dry without injury, but the washing process had to be repeated three times instead of twice. The *Beroe* values on the other hand are not in such good agreement. This is probably in part due to the animals excreting a considerable amount of slime into the water, which there undergoes slow oxidation, with evolution of carbonic acid. Thus in some experiments with *Beroe*, the water was run from the animal chamber into a stoppered bottle, and the gases in it analysed the next day. All the values thus obtained had to be rejected, as the respiratory exchange appeared to be sometimes more than twice as great as in those determinations in which the water was analysed immediately on cessation of the experiment.

The other Cœlenterata experimented on, the Medusa *Æginета* and the Siphonsphoron *Forskalia*, were obtained on only one occasion in each case, and hence it was not possible to determine the relation of their respiratory activity to temperature.

Observations on Mollusca.

Inasmuch as in the Mollusca the animal tissues are as a rule much more highly differentiated than in the Cœlenterata, the measurement of their respiratory exchange is not of so much interest from the point of view on which this research was carried out. Nevertheless sufficient observations were made in the case of *Pterotrachea* and *Octopus* to determine the relation of respiratory activity to temperature. In Figs. 7 and 8

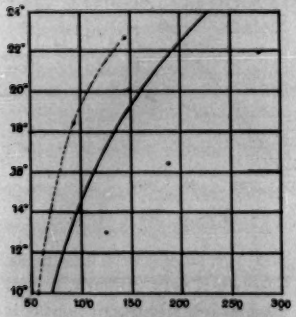

Fig. 7. Pterotrachia coronata.

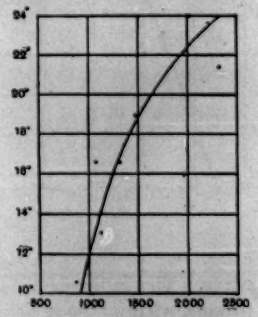

Fig. 8. Octopus vulgaris.

are shown the values obtained. Seven determinations were made in each case. The three determinations made with one large Pterotrachea are in very good agreement with each other, whilst the four made with two other small ones are also in agreement, but, as in the case of *Rhizostoma*, the respiratory activity of the small individuals is very much greater than that of the large one. All the observations on *Octopus* were made on the same animal, and hence the various values are in fair agreement with each other. In the case of the Gasteropod *Tethys*, the observations made on an individual weighing 207 grams show that its respiratory activity was considerably greater than that of one weighing only 114 grams. This latter animal had however lost all its appendages, and so probably was not in nearly such good condition.

Only one observation was made on the Pteropod *Cymbulia*, and as the animals were moribund at the time of the experiment, the value arrived at is probably too low.

Observations on Chordata and Vertebrata.

The Chordata examined consist of two Tunicates, the small *Salpa pinnata* and the large *Salpa tilesii*, and of *Amphioxus*. In Figs. 9 and 10 are shown the values obtained in the case of the Salpæ, eight

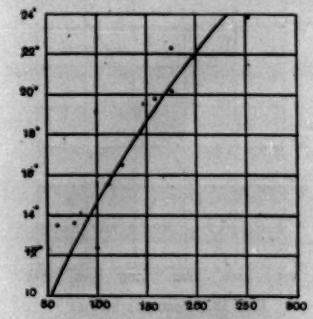

FIG. 9. Salpa pinnata.

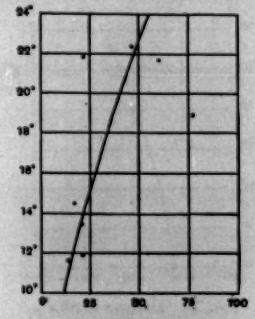

FIG. 10. Salpa tilesii.

observations being made in each case. It will be seen that they are in fair agreement with the mean curves, especially in the case of *Salpa pinnata*. These two curves show strikingly how greatly the respiratory activity of two morphologically closely allied animals may differ. Thus

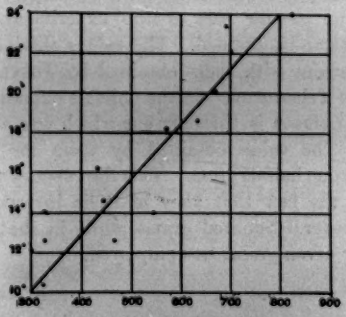

FIG. 11. Amphioxus lanceolatus.

the respiratory activity of the small *Salpa pinnata* is nearly four times as great as that of the large *Salpa tilesii*. In Fig. 11 are shown the values obtained for the respiratory activity of *Amphioxus*. As in the case of *Carmarina*, the mean curve of respiratory activity seems to be best represented by a straight line.

In Figs. 12 and 13 are shown the values obtained with the teleost fish *Heliases* and *Serranus*, seven observations being made in the one

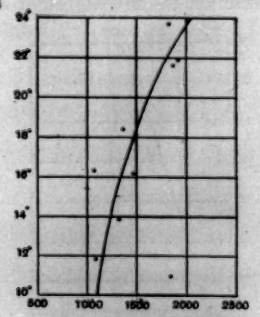

FIG. 12. Heliases chromis.

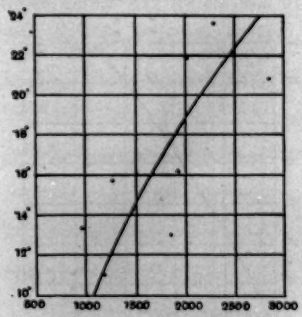

FIG. 13. Serranus scriba.

case and eight in the other. All the observations were made in each case on the same individual animals, but the different values are not in such good agreement as might be expected. This is probably due to the varying activity of the fish in the different experiments. Thus in the observation made on *Heliases* at 11°, the fish was in a state of violent activity during the greater part of the experiment, and hence an abnormally high value is obtained. The values obtained for these fish are in good agreement with those obtained by Jolyet and Regnard for other species of Teleosteans. Of the animals experimented on in the present research, *Octopus* is the only one which was also examined by these observers. The value obtained by them for the oxygen absorption is only about half as great. This discrepancy may probably be accounted for by the fact that they in their investigations used an animal weighing several hundred grams, while in the present instance one weighing only seven grams was employed.

Tabulation of Results.

We have thus far only examined the relations of the values obtained for the different animals to each other, with a view to obtaining mean curves representing the relation of the respiratory activity to temperature. In order to be able to compare together the results obtained for the different animals, the mean values for the respiratory activity, as shown by the mean curves, are given in the subjoined table. The oxygen absorbed in decimilligrams per kilogram per hour is given, at temperatures from 10° C. to 24° C. Complete sets of values are given in the case of the twelve different species of animals on which several different determinations were made, though in one or two instances at the extreme temperatures no actual observations were made. These twelve animals are arranged as nearly as possible in order, according to their respiratory activity.

Name of Animal	10°	13°	14°	16°	18°	20°	22°	24°	Relative Respiratory Activity at 16°
Salpa tilesii	12	16·5	215	27·5	34	40	47	55	1·00
Cestus veneris	17	23	30	37·5	45	53·5	63	74	1·36
Beroe ovata	29	42	56	72	88·5	106	126	147	2·62
Carmarina hastata	57	67	77	87	97	107	117	127	3·16
Rhizostoma pulmo	54	69	85	103	124	147	172	199	3·75
Pterotrachea coronata	71	82	95	112	133	159	191	227	4·07
Salpa pinnata	53	72	93	116	142	169	199	231	4·22
Tethys laporina	131	140	151	165	183	207	236	276	6·00
Amphioxus lanceolatus	296	368	439	511	583	655	727	798	18·6
Octopus vulgaris	915	1000	1115	1240	1410	1630	1935	2315	45·1
Heliases chromis	1095	1160	1230	1330	1465	1615	1795	2035	48·4
Serranus scriba	1075	1235	1430	1660	1900	2160	2440	2770	60·4
Cymbulia peronii		71							—
Forskalia contorta				172					6·25
Ægineta flavescens				288			394		10·5
Collozoum inerme	(167)			1113					40·5
Rana temporaria	1040	1120	1180	1230	1310	1500	1780	2090	44·7

The Tunicate *Salpa tilesii* has the smallest respiratory activity, that of the Ctenophore *Cestus* being about a third as much again. That of *Beroe*, the other Ctenophore examined, is about twice as great as that of *Cestus*. The Medusæ *Carmarina* and *Rhizostoma* come next, and then the Mollusc *Pterotrachea*, which has about the same respiratory activity as *Salpa pinnata*. Then with a considerable rise comes *Tethys*, and then with a still greater rise *Amphioxus*. The respiratory activity of *Amphioxus* is about a mean between those of the

pelagic Cœlenterata on the one hand, and of the teleost fish *Heliases* and *Serranus* on the other. The Mollusc *Octopus* is seen to have almost as great a respiratory activity as the fish, and to be in no way allied in this respect to the other Molluscs, *Pterotrachea* and *Tethys*.

Of the other animals examined, where only a few observations were made, the greatest interest attaches to the determination of the respiration of the Protozoon Radiolarian *Collozoum inerme*. The single determination made under normal conditions shows the respiratory activity to be nearly as great as that of the fish *Heliases* and of *Octopus*, and to be ten to twenty times as great as that of the Cœlenterata examined. It is very unfortunate that sufficient Radiolarians were not obtained to make other determinations. I have no doubt however that this single determination represents more or less closely the actual respiratory activity of this Protozoon. The water when drawn off from the animal chamber did not smell at all putrid, and hence additional oxygen could not have been absorbed from this cause. Also the gases in the water had lost 3·83 c.c. of oxygen per kilogram, so that even if a considerably larger experimental error than usual had been made in the determination, an error of only a few per cent. in the actual value would have resulted. One other determination of the respiratory exchange of this Protozoon was actually made, but there being no grounds for concluding that the respiratory activity would be so enormous, the experiment was continued so long that the oxygen in the water was almost entirely used up, whilst the Radiolarians were killed and began to undergo decomposition. The oxygen absorbed under these conditions was about a quarter of what we should expect from the normal determination, but the carbonic acid excreted was considerably larger, a quotient of 1·6 being obtained. Of the other animals examined, the two determinations made on the very small Medusa *Æginета* show its respiratory activity to be greater than that of any of the Cœlenterata and Mollusca examined except *Octopus*. The respiratory activity of the Siphonophoron *Forskalia* appears to be very large also, but this value is probably too great, as in endeavouring to drain the animals dry for weighing, they broke up into small pieces, some of which, passing through the filter-paper, escaped unweighed. At the bottom of the table, for the sake of comparison, are given the means of the values obtained by the author in a previous paper[1] for the oxygen absorbed by normal frogs. These numbers are calculated from the

[1] This *Journal*, XVII. 277. 1894.

values for the carbonic acid discharge and the respiratory quotients given in this paper. We see that the respiratory activity, as measured by the oxygen absorbed, is about the same as that of *Octopus* and *Heliases*.

In the last column of the table are shown the relative respiratory activities of the different animals at 16° C., compared with that of *Salpa tilesii* taken as unity. Here we see that the respiration of *Serranus*, which has the highest value, is 60·4 times greater than that of the Salp, whilst that of the Radiolarian *Collozoum* is 40·5 times greater. The mean respiratory activity of the four Cœlenterates, *Beroe*, *Cestus*, *Carmarina* and *Rhizostoma*, is 22·2 times less than that of *Serranus*. It is evident that the figures in this table must dispose completely of the idea that the respiratory activity of different transparent pelagic animals might be within the limits of experimental error constant.

The Effect of Temperature on the Respiratory Activity.

We have seen that with all the animals examined, the respiratory activity is considerably increased with increase of temperature. The question to be inquired into is whether the respiration of all the animals we have examined is influenced to practically the same degree by temperature, or whether, if it is affected to different degrees, there is any observable correlation between the structure of the animal and the influence produced. The effects of temperature can be more easily studied if the differences in the absolute respiratory activity of the various animals be eliminated. This is done in the following table, where the respiratory activity of each animal at 16° C. has been taken as unity, and the respiration at the other temperatures calculated as submultiples or multiples of it.

Name of Animal	10°	12°	14°	16°	18°	20°	22°	24°	Temperature Increment
Salpa tilesii	·44	·60	·78	1·00	1·24	1·45	1·71	2·00	4·5
Cestus veneris	·45	·61	·80	1·00	1·20	1·43	1·68	1·97	4·4
Beroe ovata	·40	·58	·78	1·00	1·23	1·47	1·75	2·04	5·1
Carmarina hastata	·66	·77	·89	1·00	1·11	1·23	1·34	1·46	2·2
Rhizostoma pulmo	·52	·67	·83	1·00	1·20	1·43	1·67	1·93	3·7
Pterotrachea coronata	·63	·73	·85	1·00	1·19	1·42	1·71	2·03	3·2
Salpa pinnata	·46	·62	·80	1·00	1·22	1·46	1·72	1·99	4·3
Tethys laporina	·79	·85	·92	1·00	1·11	1·25	1·43	1·61	2·0
Amphioxus lanceolatus	·58	·72	·86	1·00	1·14	1·28	1·42	1·56	2·7
Octopus vulgaris	·74	·81	·92	1·00	1·14	1·31	1·56	1·87	2·5
Heliases chromis	·82	·87	·92	1·00	1·10	1·21	1·35	1·53	1·9
Serranus scriba	·65	·74	·86	1·00	1·14	1·30	1·47	1·67	2·6
Mean	·61	·72	·85	1·00	1·17	1·35	1·56	1·80	3·3

From the mean values at the bottom of the table, we see that on an average the respiratory activity at 10° C. is only ·61, or about two-thirds that at 16° C., whilst that at 24° C. is 1·80, or nearly twice as much. With regard to the individual values, it is obvious that there is a very large variation in the different animals. Thus at 10° C. this 'temperature quotient' varies from ·40 to ·82, whilst at 24° C. it varies from 1·46 to 2·04. In the last column of the table are given the numbers obtained by dividing the temperature quotient of each animal at 24° C. with the corresponding quotient at 10° C. The numbers thus obtained, which may be called the 'temperature increments,' vary from 1·9 to 5·1. That is to say, if the values given in this table are correct, the respiratory activity in some animals is affected by a rise of temperature from 10° C. to 24° C. to nearly three times as great an extent as in others.

It may be questioned whether these considerable variations are not merely the result of very large experimental errors, or whether they can be held to afford conclusive evidence that the respiration is affected to different degrees in different animals. This question may be answered by classifying the animals examined according to their 'temperature increments,' as has been done in the following table.

Name of Animal	Temperature Increment	Remarks
Beroe ovata	5·1	mean 4·8	Transparent pelagic animals.
Cestus veneris	4·4	
Salpa tilesii	4·5	,, 4·4	
Salpa pinnata	4·3	
Rhizostoma pulmo	3·7	,, 3·0	
Carmarina hastata	2·2	
Pterotrachea coronata	3·2	,, 2·6	Opaque or only slightly transparent littoral animals.
Octopus vulgaris	2·5	
Tethys laporina	2·0	,, 2·7	
Amphioxus lanceolatus	2·7	
Serranus scriba	2·6	,, 2·3	
Heliases chromis	1·9	

One is at once struck by the evident relation existing between morphological structure and the influence of temperature on respiratory activity. Thus the Ctenophores, with a mean value of 4·8, are affected to the greatest extent by temperature. Next come the Salpæ, and next, with a considerable drop in their mean value, the Medusæ. *Amphioxus* and the Mollusca follow next, and lastly come the teleost fish, with a mean value of 2·3. As a general rule therefore it follows that the more highly the tissues of an animal are structurally differentiated, the less is the respiratory activity affected by temperature.

With regard to the individual numbers in this table, it is striking to notice that the two species of Salpæ examined have nearly identical numbers, or have their respiratory activity affected to practically the same extent by temperature, though the actual respiratory activity of one species is more than four times as great as that of the other. The large difference in the values of *Carmarina* and *Rhizostoma* may perhaps be due to the fact that though both animals are Medusæ, yet they show wide structural differences from each other, they belonging to the Hydrozoa and Scyphozoa respectively. Not much importance can be attached to the very low value of *Tethys*, as not enough determinations of the respiratory exchange were made to fix the relation of respiration to temperature at all accurately.

It is to be noted that all the animals in the upper half of the table, with large temperature increments, are transparent hyaline pelagic animals, whilst those in the lower half, which have considerably smaller values, are either quite opaque animals, or are only slightly transparent, and moreover are not pelagic. Whether this difference in the reaction of respiratory activity to temperature is directly connected with the fact of an animal being transparent and pelagic, or otherwise, or is merely due to the fact that the tissues of these transparent animals are as a rule less highly differentiated than those of the more opaque animals, it is impossible to say. It seems more probable that the slighter influence of temperature is only due to the increased differentiation of the protoplasmic tissue, whereby it is rendered more stable and less influenced by changes in its external conditions.

The Relation between the Respiratory Activity and the Size of Animals.

We have already seen that in the case of *Rhizostoma* and of *Pterotrachea* the respiratory activity is considerably affected by the size or mass of the animal. Jolyet and Regnard in their work on respiratory exchange came to the conclusion that, other conditions being similar, smaller animals have a relatively greater respiratory exchange than larger ones of the same species. In order to examine more clearly this relation between mass and respiratory activity in the present research, it will be necessary to tabulate all the different observations made according to the size of the animal under observation. For this purpose the temperature factor must be got rid of. This can be done with a fair amount of accuracy by dividing each value for the oxygen absorbed by the mean 'temperature quotient,' at the

particular temperature of the experiment. The mean temperature quotients given in the table a page or two back are only for the temperatures 10° C., 12° C., 14° C., etc.; so the values at intermediate temperatures were also calculated, and applied when necessary. In applying this correction, it is assumed that the respiratory activity of all the animals is affected to a similar extent by temperature, which we actually know not to be the case. As the relation of respiratory activity to temperature in the different animals has only been determined within a considerable range of experimental error, it was thought that less error would be introduced by using the same temperature correction for all the observations, whatever the animal, than by employing a different correction for each. In the following table are given the respective weights of each animal experimented on, and the corresponding average weight

Name of Animal	Number of Observations	Weight of Animal in grams	Mean Oxygen value at 16°	Extreme variations in Weight	Extreme variations in Respiration
Rhizostoma pulmo	6	62·2	·0167 grm.	1 : 1·72	2·78 : 1
,, ,,	2	69·6	·0154		
,, ,,	4	87·1	·0083		
,, ,,	7	93·0	·0106		
,, ,,	8	107·0	·0060		
Carmarina hastata	2	15·2	·0088	(1 : 1·73)	(1·14 : 1)
,, ,,	3	27·6	·0065		
,, ,,	5	30·2	·0100		
,, ,,	1	30·7	·0129		
,, ,,	2	34·5	·0085		
,, ,,	4	44·8	·0088		
,, ,,	5	54·7	·0078		
Beroe ovata	9	5·2	·0102	1 : 7·37	1·85 : 1
,, ,,	3	10·6	·0082		
,, ,,	1	32·2	·0064		
,, ,,	7	32·9	·0064		
,, ,,	4	38·3	·0055		
Cestus veneris	3	40·8	·0062	1 : 2·93	1·97 : 1
,, ,,	2	49·7	·0048		
,, ,,	3	61·7	·0047		
,, ,,	3	69·2	·0033		
,, ,,	1	115·5	·0028		
,, ,,	2	123·6	·0033		
Pterotrachea coronata	4	16·2	·0157	1 : 6·45	2·04 : 1
,, ,,	4	104·5	·0077		
Tethys laporina	2	114·4	·0117	1 : 1·81	1 : 1·65
,, ,,	3	206·9	·0193		
Salpa pinnata	4	2·6	·0129	1 : 1·69	1·40 : 1
,, ,,	2	2·7	·0111		
,, ,,	2	4·4	·0092		
Salpa tilesii	3	61·5	·0040	1 : 1·72	1·54 : 1
,, ,,	2	62·9	·0017		
,, ,,	2	106·2	·0029		
,, ,,	1	106·4	·0026		
Amphioxus lanceolatus	10	·21	·0472	1 : 1·24	1 : 1·12
,, ,,	3	·26	·0528		

of oxygen absorbed per kilogram per hour. These numbers are the means of the values for all the observations made under normal conditions on each animal. The numbers of observations thus made are given in the second column of the table. The weights given are those of the animals at the beginning of the experiments. If two or more animals were used in an experiment, the average weight is given in the table.

In the case of *Rhizostoma*, we see that an animal weighing 62·2 grams has a mean oxygen value 2·78 times greater than that of one weighing 107·0 grams. Animals of intermediate weight have intermediate values, there being only one exception to the orderly sequence of the values. In the case of *Carmarina*, the mass of the animal has apparently not so great an effect on the respiratory activity, but it nevertheless has some influence. Thus if all the series of experiments be separated into two groups, and means taken, the animals in the first half, with an average weight of 25·9 grams, have an oxygen value of ·0096 grams, whilst those in the second half, with a weight of 44·7 grams, have a value of ·0084 grams. In the case of *Beroe*, the variations in the oxygen value are well marked, as those in the weight are so considerable. There is in this instance no exception to the orderly interdependence of respiratory activity and mass. The same is true for *Cestus*, where the influence of mass is somewhat greater than with *Beroe*. In the case of *Pterotrachea*, only two series of observations were made, but as the weights of the animals used in them varied in the proportion of 1 to 6·45, the oxygen values also vary considerably, viz. as 1 to 2·04. The few observations made on *Salpa pinnata* prove its respiration to be as much influenced as in the other animals. In the case of *Salpa tilesii*, there is one marked exception to the general rule. If this be excluded, the remaining values show the influence of mass to be about the same as in other cases. Of the other animals experimented on, the same individuals were employed in each case in the observations made on *Serranus*, *Heliases* and *Octopus*, and hence in their case the influence of mass on respiration cannot be studied. It has already been mentioned that the two series of experiments made on *Tethys* are in disagreement with the rule, probably owing to the animal in one case being in bad condition. The two series of experiments made on *Amphioxus* also form a slight exception, probably owing to the same reason. Thus the smaller animals used in the one series of observations had been kept in captivity for some weeks, whilst the larger ones used in the other series were quite fresh.

We see therefore that in all the experiments made there is no valid exception to the general rule of the interdependence of the mass of animals and their respiratory activity. In the case of some animals, as *Rhizostoma*, the influence of mass is much greater than in the case of others, such as *Cestus*, *Beroe*, the *Salpæ* and *Pterotrachea*, which are influenced to about the same extent. In the case of other animals, as *Carmarina*, the influence is apparently slighter. Nevertheless in all cases the influence is considerable, and, I venture to believe, very much larger than has previously been supposed. With warm-blooded animals, it is obvious that, other conditions being equal, a smaller animal *must* have a greater respiratory activity than a larger one, in order to keep up its body temperature; but with cold-blooded animals, it is difficult to assign a reason why a similar phenomenon should present itself. Possibly the preservation of osmotic equilibrium between the tissues of the organism and the external medium may need the continuous application of vital energy, and as the smaller the animal the larger the proportionate surface exposed, a larger respiratory activity would necessarily result.

As in different individuals of the same species, the smaller the animal the larger the respiration, so it follows that in individuals of different species, the respiration will as a rule be greater in smaller animals than in larger. This contention receives striking support in the few experiments made in the present research on the respiratory exchange of the Medusa *Ægineta*, and of the Radiolarian *Collozoum*. Thus the Medusa, with an average weight of 1·6 grams, has a mean oxygen absorption of ·0271 grams, a value which is almost twice as great as that of any of the other pelagic animals examined, whilst *Collozoum*, with a weight of ·1 gram or less, has an oxygen value of ·1113 grams. The observations made on the other animals also support our contention. Thus, whilst the average respiratory activity of *Beroe* is about double that of *Cestus*, yet that of the largest *Beroe* individuals is about the same as that of the smallest *Cestus* ones, which have approximately the same weight. If therefore the influence of mass be allowed for, the respiratory activities of these two animals would be practically the same. In the case of *Salpa pinnata* and *Salpa tilesii* also, the different values are in fair accordance with the view that if individuals of the same weight in each case could be obtained, their respiratory activity would be about the same, although the actual respiration of the specimens of *Salpa tilesii* examined is only about a fourth of that of the specimens of *Salpa pinnata*. In fact it seems

probable that if it were possible to obtain animals of the same weight, the respiratory activities of *Beroe*, *Cestus* and the *Salpæ* would be nearly the same. In the case of *Carmarina* however, after making due allowance for weight, the respiratory activity is somewhat larger. With *Rhizostoma* and *Pterotrachea* it is considerably larger. In respect of these conclusions, it is to be remembered that the temperature increment, or effect of temperature on the respiratory activity, was found to be very nearly the same for *Beroe*, *Cestus*, and the *Salpæ*, whilst *Carmarina*, *Rhizostoma* and *Pterotrachea* had considerably lower values.

The Effect of the Condition of the Animals on their Respiratory Activity.

Most pelagic animals only live a few days in captivity, owing mainly to the abnormal conditions and unwholesome water in which they are placed in marine aquaria. In most cases therefore their condition and vitality rapidly deteriorate from day to day, they remaining in a moribund condition for a considerable time, and finally undergoing decomposition and disintegration. We should accordingly expect the respiratory activity of these animals to steadily decrease on keeping. In order to examine this point, all the observations made are tabulated in the subjoined table, according as they were made on the first, second,

Name of Animal	1st	2nd	3rd	4th
Rhizostoma	87	92	78	
	64	69	41	
	179	204	164	146
	110	100	111	
Mean	110	116	98	146
Carmarina	83	81	73	
	92	106	103	
	73	102		
	66	101		
Mean	79	98	88	
Beroe	59	42	61	
	64	97	108	212
	40	55	81	134
	65	116	116	
Mean	57	78	92	173

Name of Animal	1st	2nd	3rd	4th
Cestus	42	57		
	30	39		
	27	39		
	29	81		
Mean	32	54		
Salpa tilesii	30	62		
	19	39		
Mean	25	51		
Salpa pinnata	93	128		
Pterotrachea	181	183		
Tethys	164	208		
Octopus	1217	1375	1024	
Amphioxus	561	461	456	406
Heliasea	1810	1842	1269	
Serranus	1217	1375	1024	

third or fourth day of experiment. This was thought to be a better method than to arrange them according to the number of days the animals had been kept in captivity, as the subjection of an animal to the rapid warming or cooling of succeeding experiments, and the frequent removal of almost all the water from the animal chamber, in the washing out process, act as much more powerful agents in affecting the vitality, than two or three days' confinement in a tank. The numbers tabulated are the oxygen values reduced to 16° C., so that the temperature factor is got rid of. The influence of the mass of the animal still remains, as it was thought to be impossible to introduce a proper correction for it. When two or more different observations were made on the same day, the mean value is taken.

The mean values for *Rhizostoma* show apparently that the respiratory activity is slightly larger on the second day than on the first, but the difference between the numbers falls within the limits of experimental error. On the third day, there is a distinct decrease in the respiration. In the only instance in which experiments were made on a fourth and fifth day, the respiration steadily decreased from day to day, from the second day onwards. In the case of *Carmarina*, the experiments made extend over only three days. As in the case of *Rhizostoma*, the respiration seems to reach a maximum on the second day, and then to begin to fall again. The results obtained with *Beroe* are very curious and unlooked for. The respiratory activity steadily *increases* from the first day onwards, till by the fourth day it has become trebled in amount. This result cannot be due to experimental error, as with one slight exception, all four series of experiments made show the same rapid increase from day to day. In the case of *Cestus*, in each of the series of experiments made, a similar phenomenon is observed. As these animals are so delicate and short lived, it was unfortunately not possible to make observations on them for more than two successive days. The single series of observations made with *Salpa pinnata*, and the two series made with *Salpa tilesii*, also agree with those made on *Beroe* and *Cestus*. It is scarcely possible that this increasing respiratory activity is the consequence of a greater vitality of the animals, as in most instances their condition obviously deteriorated from day to day, and in the case of *Cestus*, the animal towards the end of the second day's experiments was sometimes clearly undergoing decomposition and disintegration. Once or twice indeed the water smelt quite putrid, though the animal was still making feeble movements. The Beroes on the other hand rarely if ever began to undergo putrefaction during

or soon after an experiment, but they fed on themselves so rapidly that, after being kept a few days in confinement, large holes would frequently appear in them: sometimes half the animal would thus disappear, though the remaining half seemed quite healthy. The Salpæ, like the Cesti, would sometimes begin to undergo decomposition, whilst still showing feeble swimming movements. An explanation of this phenomenon of increased respiration will be attempted later on.

To return to the discussion of the table, we see that in *Pterotrachea* the respiration is considerably decreased on the second day. The same is true for *Amphioxus*, where the respiration diminishes regularly from day to day. *Octopus*, *Heliases* and *Serranus* follow a similar rule, though the numbers do not diminish so regularly as in the case of Amphioxus. In *Tethys* the respiration apparently increases on the second day of experiment. It seems therefore that all the animals examined may be broadly divided into two classes. In the Ctenophores *Beroe* and *Cestus*, and in the Tunicates *Salpa pinnata* and *Salpa tilesii*, the respiratory activity rapidly increases from day to day on keeping the animals in confinement, whilst in the Medusæ, the Mollusca (with the doubtful exception of *Tethys*), and the Vertebrata, the respiratory activity either remains constant for the first day or two or else slowly decreases. This decrease is not however nearly so marked as the increase in the other animals.

The Respiratory Quotients.

As has already been mentioned, the carbonic acid excreted by the animals was measured, in addition to the oxygen absorbed. Owing to the large quantity of carbonic acid already present in the sea water, and to the considerable absorption of this gas by any water which distils over into the bulb of the pump, the errors in the determination were found to amount on an average to about ·3%. As the increase in the carbonic acid from the respiration of the animal generally only amounted to about 1·5 to 3·0% on the total volume of gas present, the determinations of the respiratory quotients are not so accurate as could be desired. In the table below are given all the quotients obtained in experiments made under normal conditions, they being classified according as they were obtained in the first, second, third or fourth day of experiment on the animal. In the last column of the table are given the mean values of all the quotients obtained with each animal, whilst in the other columns are given the means of all the values obtained on each day.

Name of Animal	1st day	Mean	2nd day	Mean	3rd day	Mean	4th day	Mean	Means of all determinations
Rhizostoma	1·38, 1·24, ·37, ·97	·89	1·51, ·63, ·92, ·61	·83	1·37, 1·03, ·87	·92	1·44, ·86	1·15	·90
,,	·90, ·82, ·87, ·81		·85, ·83, ·42		·33, 1·21, ·70				
,,	·74, ·81		·81, ·90						
Carmarina	1·46, 2·06, 1·95, 1·34	1·04	1·80, 1·10, ·05	1·08	2·16, 1·21, ·74	1·37			1·10
,,	1·23, ·78, 1·21, ·66		1·02, ·84						
,,	·53, ·21, ·90, ·84, ·36								
Beroe	·98, ·91, ·83	1·00	1·02, ·37, ·76	·82	·77, ·95, 1·15	·96	·78, ·80	·81	·84
,,	1·79, 1·87, 1·16		1·00, 1·03, ·76				·85		
,,	·30, ·72, ·48								
Cestus	·24, ·74, 2·06, 1·43	·80	1·04, ·90, 1·05	·95					·79
,,	1·00, 1·05, ·30, ·80		·82						
,,	·07, ·43, ·64								
Pterotrachea	1·22, 1·41, 1·44	1·22	1·16, ·96	1·06					1·18
,,	1·12, 1·03, 1·09								
Tethys	·48, ·86, ·87, ·89	·78	·99, ·95	·97					·84
Octopus	·88, ·92, ·94	·91	·83, 1·00, 1·08	·97	1·01	1·01			·95
Salpa pinnata	·94, 2·04, ·96, ·90	1·13	1·06	1·06					1·12
,, ,,	1·13, ·96, ·93								
Salpa tilesii	·69, ·83	·76	·15, ·92	·54					·65
Amphioxus	·72, 1·19, ·91	·96	1·18, 1·29, 1·09	1·19	·71, ·97	·84	·80, ·65	·73	·96
,,	·99, ·98								
Heliasea	1·05, 1·11, ·78	·98	1·06, ·80, ·79	·88	·91, ·64, ·82	·79			·88
Serranus	1·08	1·08	·45, ·42	·44	·97, ·70, ·69	·79	·94, ·89	·92	·77
Collozoum	1·06								1·06
Ægineta	·82, ·81	·82							·82
Forskalia	·86								·86
Cymbulia	·99								·99

Of the mean quotients, we see that in three instances the numbers rise above unity. In the case of *Pterotrachea* and *Salpa pinnata*, this is probably due to the animals being in a moribund state at the time the experiments were made, so that metabolic equilibrium was not being maintained: the katabolic processes exceeding the anabolic, an abnormally small amount of oxygen would be absorbed, in proportion to the carbonic acid excreted. Thus it was never found possible to keep either of these animals alive for more than three days after they were captured. The quotient obtained with *Carmarina* is due to a few excessively high values, which raise the average value abnormally. These high values may be due largely to experimental error, as they were obtained in some of the first experiments made, when the limits of error would naturally be greater than subsequently. Of the other animals experimented on, the mean quotients fall within the limits of ·96 to ·65. They are as a whole somewhat higher than those generally obtained with warm-blooded animals. This is probably due to the fact that the animals experimented on were kept in captivity under abnormal conditions, for the water of an aquarium must needs be very unwholesome and impure compared with that of the open sea. In fact in the majority of cases the animals at the time of the experiments were slowly dying. Thus if we examine the mean quotients of the experiments made on successive days, we see that as a rule they gradually increase. In *Rhizostoma* this increase is well marked after the second day. In *Carmarina* there is a steady rise on the second and third days, whilst in *Cestus*, where the animal was as a rule obviously in a moribund condition on the second day of experiment, there is a considerable rise in the mean quotient. In *Beroe* on the other hand there is no apparent increase in the quotient. With the other animals experimented on, not enough observations were made after the first day of experiment to properly determine whether the quotients rise or keep constant. The low quotient obtained with *Salpa tilesii* is due to an experimental error, arising in the following way. The animals experimented on were sometimes transferred direct from the jars in which they had been placed when caught by the fishermen, into the animal chamber. Now these jars were filled with fresh sea water, which contained less than two-thirds as much carbonic acid as the aquarium tank water, which was used in the experiments. As has been mentioned, the respiration chamber containing the animal was washed out several times before the experiment was begun. About twenty minutes was generally allowed for the process, so that the water, which probably contained different

amounts of the gases, might soak out of the tissues of the animal. The time allowed seemed to be sufficient in almost all other cases, but with *Salpa tilesii*, though half-an-hour or more was generally allowed, the somewhat horny exterior of the animal seemed to delay the soaking out process considerably. Thus if the animal had been transferred directly from the pure sea water, the retention of about 2 % of the original water in its tissues would be sufficient to diminish the apparent carbonic acid excretion almost to zero. In fact, in a few instances, this result was actually obtained. The oxygen values would be affected only slightly by this source of error, as the pure sea water only contained about 2 c.c. more oxygen per kilogram than the tank water, and so an error of not more than about ·04 c.c. would be introduced.

It might be thought that the somewhat high quotients obtained are accountable to the diminished tension of the oxygen in the water towards the end of an experiment. Thus as the tension diminishes, it becomes more difficult for the tissues of the animal to absorb it, and so less oxygen might be taken up, whilst the carbonic acid excreted remained the same. If this is the case, then it follows that on an average the less the tension of the oxygen, the greater will be the quotient. The sea water employed contained as a rule about 4 c.c. of oxygen per kilogram. In most experiments about 2 c.c. of this was absorbed, but in some, more than 3 c.c. In the following table are given the mean values of all the quotients obtained, with the different volumes of oxygen present.

Oxygen per kilogram	·0 to ·5 c.c.	·5 to 1 c.c.	1 to 2 c.c.	2 to 3 c.c.	3 to 4 c.c.	over 4 c.c.
Number of observations	17	7	35	63	44	9
Mean quotient	1·44	·93	·93	·95	·93	1·02

We see that where there is more than ·5 c.c. of oxygen per kilogram left in the water, the quotient remains practically constant. The higher quotient obtained when more than 4 c.c. were present, is due to one or two abnormally high values, which raise the average considerably. When on the other hand the oxygen falls below ·5 c.c., and when in most instances the animal was asphyxiated, the quotient is considerably increased. These cases of asphyxiation we will now proceed to discuss.

The Respiratory Exchange during and after Asphyxia.

It was noticed by Spallanzani, at the beginning of the present century, that animals such as frogs, confined in an atmosphere of nitrogen, continued to exhale carbonic acid to almost as great an

extent as when they breathed air. Paul Bert[1] found that newly born rats and kittens continued making respiratory movements for twenty or thirty minutes, if placed in an atmosphere of nitrogen. They must therefore have continued to expire carbonic acid, though they were absorbing no oxygen. He also confined various mammals and birds in small volumes of air, and after the animals had died of asphyxiation, determined the composition of the gas in the chamber. In these cases, though as a rule only about two to four volumes of the oxygen in the air were left, yet the amount of carbonic acid expired, in proportion to the oxygen absorbed, was scarcely if at all greater than under normal conditions. It was thought to be of interest to make observations on the respiratory exchange of the lower marine animals during asphyxia, in order to observe to what extent these animals can continue to excrete carbonic acid, when all or nearly all of the oxygen in the water has been used up. In the table below are given all the results obtained. One or two experiments are also included, in which

Name of Animal	Mean respiratory quotient			Oxygen absorbed in grams per kilo per hour at 16°			Oxygen left in water at end of experiment	Remarks
	normally	during asphyxia	after asphyxia	normally	during asphyxia	after asphyxia		
Rhizostoma	·90	·87	·83	·0103	·0122	·0186	·43 c.c.	Animal remained
,,	·90	1·13	1·47	·0103	·0036	·0089	·11	motionless for some
Carmarina	1·10	·95	—	·0087	·0121	—	·27	hours after experi-
,,	1·10	·98	·81	·0087	·0057	·0101	·26	ment, but had re-
Beroe	·84	2·00	2·05	·0072	·0067	·0116	·03	covered next day.
,,	·84	2·47	—	·0072	·0059	—	·00	
Cestus	·79	·88	—	·0038	·0030	—	·38	
Salpa pinnata	1·12	1·64	—	·0116	·0057	—	·06	
Salpa tilesii	·62	2·46	1·36	·0028	·0036	·0091	·00	
Colloconm	1·06	1·59	—	·1113	·0265	—	·37	
Octopus	·95	1·18	—	·1240	·0421	—	·37	
Amphioxus	·96	1·08	·81	·0511	·0262	·0491	·39	All the animals re-
								covered a few hours
								after the experi-
								ment.
,,	·96	2·17	—	·0511	·0081	—	·29	All the animals were
								alive at the end of
								the experiment.
,,	·96	4·45	1·30	·0511	·0055	·0166	·00	5 out of the 120 in-
								dividuals were alive
								at the end of the
								experiment.
Heliases	·88	1·07	2·58	·1330	·0934	·0086	·31	
Serranus	·77	·88	—	·1660	·1077	—	·82	3 out of the 5 fish
,,	·77	1·07	1·54	·1660	·1076	·0121	·36	killed.

[1] *Leçons sur la Physiologie comparée de la Respiration*, 510. 1870.

the asphyxiation did not proceed far enough to kill the animals. These cases are remarked upon in the last column of the table. In all other instances, the animals showed no signs of vitality after the termination of the experiments. In experiments on asphyxia with marine animals, it should be noted that the conditions are less complicated than in similar experiments made with air breathing animals. The phenomena observed are due solely to the want of oxygen, and not at all to the deleterious influence of the carbonic acid excreted. Thus by reason of the large quantity of salts present in the sea water, the tension of the carbonic acid is so slight as to be almost negligeable, whether the water contain 70 c.c. per kilogram, the amount usually present in the tank water, or 88 c.c., the largest amount present in any of the experiments made on asphyxia.

In the table are given in the case of each animal the mean respiratory quotient obtained under normal conditions, the quotient obtained whilst the animal was undergoing asphyxiation, and in several instances, the quotient of the animal after asphyxiation. The amounts of oxygen absorbed under these three conditions are also given. In the last column of the table but one are shown the volumes of oxygen still remaining in the water in which the animal was asphyxiated. This was generally about ·3 c.c., but in five experiments it sank to ·06 c.c. or less. With regard to the respiratory exchange during asphyxia, we see that in the majority of instances quotients considerably greater than unity were obtained. In four instances they reach above 2·0, and in one instance above 4·0. It is evident therefore that marine animals can go on excreting carbonic acid, when little or no oxygen can be absorbed. It is not altogether to be inferred from Spallanzani's experiments on frogs that this would be the case, for in these animals there is a large amount of oxygen stored up in the blood, whilst in the lower marine animals there is probably no store at all to draw upon. The nearly normal quotients obtained in a few cases are due to the experiments not being continued long enough. We have already seen that when the amount of oxygen present in the water remains above ·5 c.c. per kilogram, the quotient keeps perfectly normal; and from these results it seems that it is only when the oxygen present sinks to about ·4 c.c., or less than a tenth of its ordinary value, that the quotient is affected.

As would be expected, the respiratory quotients of the animals after asphyxiation are considerably greater than unity, though of course the amount of oxygen present in the water was as great as in normal ex-

periments. It is instructive however to compare the amounts of oxygen absorbed by the asphyxiated animals belonging to the different classes of the Animal Kingdom. In the pelagic animals, such as the Medusæ and *Salpa tilesii*, the oxygen absorbed is generally rather larger than that absorbed by the living animals under normal conditions. This is because of the processes of decomposition and putrefaction, which set in very rapidly, immediately these animals are killed; and indeed, frequently before they have lost all power of vital movement. Thus in less than twenty-four hours a living Cestus may entirely disintegrate, and the proteids of its tissues passing into solution, all traces of it disappear. With the higher animals such as teleost fish the phenomena observed are very different. Here the processes of decomposition begin very slowly; so in the case of *Heliases* and *Serranus* the amount of oxygen absorbed by the asphyxiated animals is only about a fifteenth of that absorbed under normal conditions. *Amphioxus* occupies an intermediate position, as the asphyxiated animals absorb about a third of the normal amount of oxygen. It is instructive to examine the phenomenon of death, as instanced in the transparent pelagic animals. Here the transition from the living state to the dead is so gradual that it is impossible to say when one condition ceases and the other begins. We have already mentioned the fact that these animals may be undergoing rapid decomposition, and yet continue to show vital movements. In fact their contractility may only cease on their actual disintegration. The oxygen absorption, so far from decreasing in the initial stages of decomposition, is if anything greater. The carbonic acid excretion is also greater; only with reference to the oxygen absorption it is considerably larger than in the animals when living in a state of metabolic equilibrium.

Attention must be drawn to the remarkable vitality exhibited by *Amphioxus*. In the first experiment, not one of the sixty-eight individuals used was killed, though the water only contained ·39 c.c. of oxygen per kilogram. In the second experiment, made with 120 fresh specimens, none were killed though the water contained only ·29 c.c. of oxygen, and the oxygen actually absorbed by the animals was less than a sixth of the normal amount. This experiment was immediately followed by another, on the same animals, and was continued so long that not a trace of oxygen was left in the water, and the amount of oxygen absorbed was only a tenth of the normal. The animals must therefore have been totally deprived of oxygen for some hours, and yet, on the day after the experiment, five if not more of them had recovered.

The fish *Serranus* was very different, as in one experiment three out of the five fish used were asphyxiated, though the water still contained ·82 c.c. of oxygen per kilogram.

In most of the experiments in which the animals were asphyxiated, the amount of nitrogen gas found in the water was somewhat larger than usual, it being in some instances increased by as much as 2 or 3 c.c. per kilogram. This is probably due to the decomposition, on boiling out the gases from the water, of the increased amount of urea and other nitrogenous bodies excreted into the water by the animals.

The Solid Constituents of Marine Animals.

It is obvious that, bulk for bulk, transparent pelagic animals must contain very much less solid matter in their tissues than the higher Mollusca and Vertebrata. It is of some interest to determine how much is actually present, in order to be able to compare the respiratory activity of these animals, calculated on the amount of solid constituents present, with that of the Vertebrata calculated from the same stand-point. The amount of solid matter present is so small, as to be considerably less than that of the salt in the salt water in the tissues of the animals. It is impossible to determine how much, if any, of these saline constituents in the tissues are in actual combination with the proteid constituents; and hence it was thought to be best to look upon none of them as in combination, and to determine only the amount of solid organic constituents present in the tissues. From the total solids found in an animal was therefore subtracted the amount of salt contained in a weight of water equal to that of the animal itself. This correction assumes that the organic constituents present take up no space at all in the animal. Even if this is not the case, the error thereby introduced is very small, and as it is not one that can be properly calculated for, it has been ignored.

In order to determine the solid constituents present, the animal was evaporated down nearly to dryness in a porcelain basin on the water bath, and was then dried for about forty hours at 100° C. in an air bath, when a practically constant weight was attained. As the salt present in the animal makes up as a rule all but a tenth to a twentieth of the total solid constituents, determinations of the amount of salt present in specimens of the water in which the animal had been living had to be made. As the Specific Gravity of the tank water in the Aquarium was constantly varying, determinations of the saline constituents had to be

made every few days. As in the case of the animals, the water was evaporated down, and the salt dried for about forty to fifty hours at 100°. In the table in Appendix B are given the amounts of salt found in the different determinations, and in a few cases, the Specific Gravity of the water. In the following table are given the percentages of solid matter found in the different animals. The determinations are arranged in different columns, according to the length of time the animals had been kept in captivity. As has already been mentioned, pelagic animals decrease very rapidly in size on keeping. This may be due in part to the shrinking of the tissues of the animal, with expulsion of water, or entirely to the actual wasting away of the tissues. In the one case, the percentage of solid constituents would increase, and in the other remain constant.

Name of Animal	0 to 3 days	4 to 6 days	7 to 10 days	over 10 days	Mean percentage of solid constituents
Cestus	·25, ·29, ·23, ·22, ·25, ·18				·24
Salpa pinnata	·29, ·21, ·29	·29			·26
Carmarina	·35, ·28, ·28	·50, ·38, ·44	·42		·38
Collozoum	·37, ·42				·40
Salpa tilesii	·44, ·35, ·29	·64			·43
Rhizostoma	·51	·47	·72, ·41		·53
Pterotrachea	·66, ·56	·37			·53
Forskalia	·56				·56
Beroe	·69, ·59, ·42	·41		·88	·60
Cymbulia	·66				·66
Tethys	(4·23) 1·20				1·20
Octopus				11·7	11·7
Amphioxus		12·5		13·1	12·8
Serranus				17·2, 16·2	16·7
Heliases				22·3	22·3
Rana temporaria					19·1

In consequence of the very small amount of organic matter present in the total solid constituents in proportion to the saline matter, and of the difficulty of determining the exact amount of salt present in each case, the different determinations vary considerably amongst themselves. *Cestus* and *Salpa pinnata* appear to contain the smallest amount of solid matter, viz., ·24 and ·26 % respectively. The different values in their cases agree fairly well amongst themselves. Then, with a considerable rise, came *Carmarina*, *Collozoum* and *Salpa tilesii*. Then with another considerable rise, *Rhizostoma*, *Pterotrachea*, *Forskalia*, *Beroe* and *Cymbulia*. *Tethys*, which comes next, contains about double their amount. From this point there is a tremendous rise, as *Octopus* and *Amphioxus*, which come next, contain about ten times as large a proportion of solid

constituents as *Tethys*, and about fifty times as much as *Cestus* and *Salpa pinnata*. Lastly the fishes *Serranus* and *Heliases* contain about half as much again as *Amphioxus*, or respectively 70 and 93 times as much as *Cestus*. Mammalia are stated to contain about 30 % of solid constituents in their bodies, whilst their skeletal muscles, which perhaps form a better comparison with the tissues of pelagic animals, contain about 25 %. In four different determinations on frogs' skeletal muscle, the proportion of solid matter was found to be 18·9, 19·1, 18·7 and 19·6, or on an average, 19·1 %. In respect of the amount of solid constituents, we see therefore that the transparent pelagic animals show a very sharply defined difference from the Vertebrata.

With reference to the effect of captivity on the solid constituents of an animal, Carmarinæ killed after four to six days seem to contain a considerably larger proportion of solid matter than those killed after one to three days. In the case of *Beroe*, the *Salpæ* and *Rhizostoma* also, the percentage seems if anything to increase, but the determinations are so few that it is not possible to speak with certainty. In the case of *Tethys*, a very great shrinkage occurs on the death of the animal. Thus in an animal alive at the time of weighing, the proportion of solid matter was found to be 1·20 %. In another, which had died some hours before, the proportion was 4·23 %. Though no such result as this was obtained with other animals, yet we seem justified in concluding that when on being kept in captivity, pelagic animals decrease in size and deteriorate in vitality, the diminution is not due entirely to the animals wasting away, but partly to a shrinkage of the tissues.

The Respiratory Activity per dry Body Weight.

Having determined the amounts of solid constituents in the tissues of the different animals, it becomes possible to calculate the respiratory activity in grams per hour per dry body weight. This undoubtedly forms the most correct basis on which to compare the respiration of these animals, both amongst themselves, and with that of the higher animals, as the mammalia. Of the animals examined, *Heliases* contains the largest proportion of solids in its tissues, viz. 22·3 %, or 223 grams. per kilogram: so in the following table are given the amounts of oxygen absorbed per hour at 16°, by such a body weight of an animal as would contain 223 grams of solid organic constituents. At the end of the table, for the sake of comparison, are given the amounts of oxygen absorbed per kilogram body weight per hour by man and by dogs. These

numbers are calculated from the tables given by Munk[1]. As in these instances the tissues contain about 30 % of solids instead of 22·3 %, the values ought to be reduced by nearly a third, when comparing them with the other values in the table. The number given for frogs is calculated on the same basis as for the other animals.

Name of Animal	Oxygen absorbed per 223 grm. dry tissue	Average weight of animal	Name of Animal	Oxygen absorbed per 223 grm. dry tissue	Average weight of animal
Collozoum	6·2050 grm.	·1 grm.	Octopus	·2363 grm.	7·1 grm.
Salpa pinnata	·9949	3·2	Beroe	·2342	23·8
Forskalia	·6849	15·9	Serranus	·2217	4·1
Carmarina	·5106	34·0	Salpa tilesii	·1426	84·4
Pterotrachea	·4712	60·4	Heliases	·1330	10·4
Rhizostoma	·4334	83·8	Amphioxus	·0890	·24
Cestus	·3484	76·7	Man	·4167	
Tethys	·3066	160·7	Dog	1·1821	
			Frog	·1436	

One is chiefly struck, on looking at this table, by the very great respiratory activity of the transparent pelagic animals, when it is calculated according to dry body weight. Thus in most cases it is greater than that of man, in *Salpa pinnata* it being about three times, and in the Radiolarian *Collozoum* nearly twenty times as great! When we remember that these values are for a temperature of 16°, whilst in man it is for one of 38°, the metabolism of these lowly animals seems truly astounding. When compared with the respiratory activity of the frog that of the pelagic animals also appears very great. In most cases it is about three times, but in that of *Collozoum* actually forty-three times as large! The small value for *Amphioxus* is perhaps to be expected when we consider the sluggish nature of these animals.

In the third column of the table are given the average weights of the animals used in all the experiments. We see that as the respiratory activity steadily decreases in the different animals, their average body weight steadily increases. Of the transparent pelagic animals, there is only one marked exception to the orderly interdependence of the values for the respiratory activity and mean body weight, viz., in the case of *Beroe*. Here the respiratory activity is less than half of what we should expect. The value for *Salpa tilesii* is also somewhat low. The fact that the weight of the *Heliases* individual used in the experiments was two and a half times as great as that of the *Serranus* ones, may perhaps be the chief cause of its respiratory activity being so much smaller.

[1] *Physiol. des Menschen und der Säugethiere*, 82. 1888.

As a general rule therefore, amongst animals of the same class, it would seem that the respiratory activity depends chiefly upon two attributes, the mass of the animal, and the amount of solid constituents in its tissues.

The Effect of Captivity on the Size of Pelagic Animals.

Most pelagic animals when kept in aquaria never take food, but use their own tissues as their source of vital energy. In consequence of this, even if they otherwise remain healthy, they rapidly dwindle away in size. This very rapid diminution was not sufficiently appreciated in the first part of the experiments made, and it was thought enough to weigh the animal at the end of the series of experiments, in order to be able to calculate the respiration per kilo body weight. In the latter two-thirds of the experiments, however, the animals were weighed before as well as after the experiments, and generally at intermediate times as well, in order to determine the loss of weight. In performing this weighing, the animal as a rule was allowed to drain free from water on a porcelain sieve for half-a-minute, and was then weighed as rapidly as possible in a basin or beaker. In the case of *Cestus*, the animals were too delicate to permit of this treatment, so as much of the water as possible had to be sucked with a pipette out of the jar containing the animal to be weighed. In order to determine as exactly as possible the average daily loss in weight of pelagic animals in confinement, a good many weighings were made on animals other than those used for the respiration experiments. The weighing was generally made only once in three days, as the process is rather a strain on the animal. The average daily percentage loss in weight could then be easily calculated by logarithms from the loss over a period of several days. The weighings were generally made at the same hour on each day, but if not, the difference in the time was always calculated and allowed for. In the table are shown the daily percentage losses found in the different animals. If the weighings were continued on the same animal for more than a fortnight the daily percentage losses are given in a second line in the table, underneath the first. In the last column of the table are given the percentages of the final weights of the animals on their original weights. In another column is given the average temperature of the water in which the animals were kept during each experiment. It generally only varied two or three degrees, but in some cases more. These temperature variations doubtless account for part of the great variations observed in the loss in weight from day to day.

Name of Animal	Date of experiment	Original weight of animal in grams	Temperature of water	Percentage decrease in weight during day the													Percentage of final weight on original weight
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	
Rhizostoma	Jan. 16	62·7	13·0°	7·4	7·4	7·4	7·4	7·4	7·4	7·2	7·2	7·2	10·7	10·7	10·7	9·7	9·7	11·61
,,	16	62·7	13·0	9·7	3·8	3·8	3·8	3·8	2·3	2·3	2·3	5·4	5·4	5·4	5·4	4·7	4·7	
,,	16	62·7	13·0	4·7	4·7	2·9	2·9	2·9	2·9	2·9	2·9							
,,	22	167·2	13·0	9·5	9·5	9·5	6·1	6·1	6·1	8·1	8·1	8·1	9·9	9·9	9·9	9·9	10·4	7·02
,,	22	167·2	13·0	10·4	10·4	6·8	6·8	6·8	6·8	5·8	5·8	5·8	5·8	10·0	10·0	10·0	10·0	
,,	23	211·8	12·6	12·5	12·5	12·5	6·9	6·9	6·9	6·1	6·1	6·1	6·2	6·2	6·2			31·92
,,	Feb. 5	91·2	10·8	6·7	6·7	6·7	2·3	2·3	2·3	2·3								74·23
,,	12	141·9	11·0	11·1	11·1	11·1	7·0	7·0	7·0	7·0	6·7	6·7	6·7	6·7	5·2	5·2	5·2	19·84
,,	12	141·9	11·0	5·2	5·4	5·4	5·4	3·9	3·9	3·9	9·0	9·0						
,,		(First fortnight)	(Mean)	9·4	9·4	9·4	5·9	5·9	5·9	6·1	7·0	7·0	8·4	8·4	8·0	8·3	8·4	
,,		(Second fortnight)	(Mean)	8·4	6·5	5·3	5·3	4·8	4·3	4·0	5·7	6·7	5·6	7·7	7·7	7·4	7·4	
Carmarina	Jan. 22	46·0	13·0°	25·1	25·1	25·1	10·6	10·6	10·6	5·1	5·1	5·1	6·9	6·9				22·17
,,	28	44·9	11·0	12·3	12·3	8·7	8·7	8·7	8·6	8·6								49·89
,,	28	40·4	11·0	24·3	24·3	13·3	13·3	13·3	9·3	9·3								32·07
,,	Feb. 5	42·3	10·8	15·4	15·4	15·4	20·8	20·8	20·8	31·4	31·4	31·4						9·74
,,	5	35·0	10·8	17·1	17·1	17·1	19·0	19·0	19·0									30·29
,,	23	47·5	11·0	14·1	14·1	14·1	15·1	15·1	15·1	18·3	18·3	18·3						21·18
,,	23	33·9	11·0	9·3	9·3	9·3	15·4	15·4	15·4	15·0	15·0	15·0						27·77
,,	26	74·2	11·5	11·6	11·6	11·6	10·6	10·6	10·6	13·6	13·6	13·6						31·93
,,			(Mean)	16·1	16·1	14·3	14·2	14·2	13·7	14·5	16·7	16·8	6·9	6·9				
Beroe	Jan. 22	34·2	15·0°	16·7	16·7	15·4	15·4	8·1	8·1	8·1	5·6	5·6	5·6	5·6	5·6	5·6	8·6	21·75
,,	22	34·2	15·0	8·6	8·6													
,,	Feb. 6	62·7	14·0	11·5	11·5	17·1	18·1	18·1										34·37
,,	12	16·2	10·0	23·5	23·5	18·4	18·4	18·4	18·4	18·4	18·4							16·60
,,	20	64·2	8·5	8·6	8·6	8·6	14·5	14·5										55·76
,,	26	39·1	11·5	16·6	12·7	12·7	12·7	9·2	9·2	9·2	7·1	7·1						18·06
,,			(Mean)	15·4	14·6	14·4	15·8	13·7	11·9	11·9	10·4	6·4	5·6	5·6	5·6	5·6	8·6	

Name of Animal	Date of experiment	Original weight of animal in grams	Temperature of water	Percentage decrease in weight during day the													Percentage of final weight on original weight
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	
Cestus	Jan. 28	137·0	10·5°	9·3	9·3													82·33
,,	Feb. 5	121·0	10·2	19·0	19·0													65·04
,,	7	60·7	12·3	17·8	17·8													67·55
,,	19	122·8	8·0	9·7	9·7	9·7												74·59
,,	23	174·4	9·0	9·3	9·3	9·3												74·66
,,			(Mean)	13·0	13·0	9·5												
Pterotrachea	Jan. 28	14·3	16·0°	37·7	35·4													46·36
,,	29	24·7	16·0	23·0														85·87
,,	Feb. 22	105·6	10·0	18·8														78·38
,,	26	128·7	11·0	27·9	31·4													48·64
,,			(Mean)	26·9	33·4													
Salpa pinnata	Jan. 22	21·2	16·0°	28·0	28·0													51·89
,,	Feb. 15	18·4	13·0	17·6	8·3	8·3												67·82
,,	15	24·1	13·0	21·0	7·9	7·9												65·06
,,			(Mean)	22·2	14·7	8·1												
Collozoum	Feb. 19	2·19	15·5°	38·5														61·46
Æginета	22	15·1	17·0	60·3														39·73
Octopus	2	7·1	15·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0				89·44
Tethys	9	206·9	11·0	100	100	118	118	118	128	128	128	128	128	131	101	101	101	149·0
,,	9	206·9	11·0	101	110	110	110	110	119	119	119	119	123	123	149	149	149	

In the case of *Rhizostoma* the average loss is about 8 % per day but even the mean numbers vary considerably. The loss seems to only decrease slightly after the observations have been carried on three weeks or more. These Medusæ could be kept alive in captivity longer than any other pelagic animals, one of the recorded experiments lasting over five weeks. At the end of this time, the animal was only 11·6 % of its original weight. In another case the animal dwindled down to only 7 % of its original weight. Carmarinæ as a rule could not be kept alive more than a week or ten days. During this time they lost on an average about 15 % per day, though, as can be seen in the table, in the extreme observations the amount varied from 5 % to 31 %. In consequence of this great daily loss, the animals at the end of the experiments generally weighed only about a fifth of their original amount. In the case of *Beroe*, the animals decreased daily about 15 % in weight during the first four or five days, and then seemed to lose gradually less and less. Insufficient experiments were made, however, in which the experiments could be prolonged for more than a few days, to test this point decisively. In the case of *Cestus*, the animals could not be kept alive for more than three days, and also it was never possible to weigh them more than twice, as the second time they were always torn in pieces, however great care was observed in the process. Their daily loss in weight was about 12 %. In the case of *Salpa pinnata*, the loss in weight seems to diminish from day to day, it being apparently about 22 %, 15 % and 8 % respectively on the three days it was observed. *Pterotrachea* is even shorter lived than *Cestus* and *Salpa pinnata*, as these animals could not be kept alive for more than two days. During this time the daily loss was about 30 %, or nearly a third of the weight of the animals. The single observation made on the Protozoon *Collozoum* showed a loss of in one day 38 %; whilst the Medusa *Æginета* showed the enormous loss of 60 %, or nearly two-thirds of the body weight of the animal. The Medusæ were all alive after this weighing, but the next day they were dead and had almost entirely disintegrated, so the experiment could not be continued. The single observation made with *Octopus* seemed to show a daily loss of 1 %, but this observation is not of much value, as the animal can vary the amount of water taken into its body. The observations made on *Amphioxus*, *Serranus* and *Heliases* seemed to show no decrease at all in weight; but the slight actual loss would probably be hidden or exaggerated by the errors arising from different amounts of water clinging to the surface of the animals in the different weighings. *Tethys* is a

remarkable animal, as it changes its weight considerably from day to day, and never remains constant. The numbers given in the table show its daily weight, relative to that at the beginning of the experiment taken as 100. We see that after being kept a month, the animal weighed half as much again as at first.

It has been stated that these frequent weighings of the animals used were only made in the latter two-thirds of the experiments. In these cases the average weight of the animal during each experiment was thus determined directly to within about 3 %, and so only small corrections had to be applied. In the former experiments, larger corrections had to be calculated. The values used in these calculations for the daily loss in weight of the animals are those mentioned above, viz. 8 % for *Rhizostoma*, 15 % for *Carmarina* and *Beroe* and so on. In the case of *Salpa tilesii*, unfortunately no specimens were obtained after the weighing experiments were commenced; hence, in calculating the weights of these animals during the experiments, it was assumed that the loss per diem was 10 %. In the single experiments made with *Cymbulia* and *Forskalia*, a similar correction was adopted.

It may be questioned whether the enormous daily decrease in weight of pelagic animals we have just noticed is a normal process, which would take place in these animals in the open sea under natural conditions, if they were deprived of food, or whether it is a pathological one, engendered by the abnormal circumstances under which they are placed in captivity. Now the percentage of solid matter in the tissues of these animals has been determined, and we may assume for the present that this solid matter consists practically of proteid matter. As we also know the average respiratory activity of these animals, it is possible to calculate what the daily loss in weight of these animals should be during starvation. The values thus calculated ought to be very nearly the same as those found by direct weighing, supposing that all the tissue of the body on which the animal lives is fully oxidised to carbonic acid, water and urea or uric acid, as is very nearly the case in the healthy animal body. The composition of the solid matter is assumed to be: carbon, 53 %; hydrogen, 7 %; nitrogen, 16 %; oxygen, 23 %, and sulphur, 1 %. 100 grams of proteid of this composition, oxidised to carbonic acid, water, urea and sulphuric acid, requires 148·36 grams of oxygen, and gives 169·2 grams of carbonic acid. In the table is shown the daily percentage loss in weight of each animal, as calculated from the respiratory activity

of the animals at their mean temperature during the weighing experiments.

Animal	Mean temperature of experiments	Respiratory activity	Percentage of solid constituents	Actual daily percentage loss in weight	Calculated daily percentage loss in weight
Rhizostoma	12·0°	·0069 grm.	·53	8·0	2·1
Carmarina	11·0	·0062	·38	15·0	2·7
Beroe	12·0	·0042	·60	15·0	1·1
Cestus	10·0	·0017	·24	12·0	1·2
Pterotrachea	13·0	·0088	·53	30·0	2·7
Salpa pinnata	14·0	·0093	·26	22·0	5·8
Octopus	15·0	·1170	11·7	1·0	1·6
Collozoum	16·0	·1113	·40	38·5	45·2
Tethys	16·0	·0165	1·20	—	2·2
Salpa tilesii	16·0	·0028	·43	—	1·0
Amphioxus	16·0	·0511	12·8	—	·7
Serranus	16·0	·1660	16·7	—	1·6
Heliasea	16·0	·1380	22·3	—	1·0

As compared with the actual daily loss in weight, these calculated values are very small. In the case of *Beroe*, *Cestus* and *Pterotrachea* they are only about a tenth as much, and in *Rhizostoma*, *Carmarina*, and *Salpa pinnata* about a quarter as much. Only in the case of *Collozoum* and *Octopus* are the calculated and the actual values more or less the same. How then is this enormous difference to be accounted for? In the first place, the percentages of solid constituents found are probably somewhat too high, as the tissues were dried only at 100°. In order to drive off all traces of moisture, a temperature of 110° to 120° is necessary; but it was impossible to heat them to this temperature, as unless a very nearly constant temperature is maintained in all cases, varying proportions of water are driven off from the salt in the tissues, and the determinations rendered valueless. Again, we have seen that in all probability the loss in weight of pelagic animals, on being kept in captivity, is not due entirely to their wasting away, but partly to a shrinkage of the tissues. Still again, the solid organic constituents may not consist by any means of pure proteid. It does not however seem probable that these sources of error can account for nearly the whole of the immense difference existing between the theoretical and actual values. It seems more likely that considerable portions of the tissues of the animals pass away into solution in the water unoxidised. It may be remembered that in the experiments made on *Beroe*, whenever the water from the animal chamber was kept over night before analysis, the apparent respiratory exchange was found

to be much larger, it being frequently doubled. In such cases the water generally smelt putrid. In the few instances with other animals in which the water was kept before analysis, the respiratory exchange seems as a rule to be somewhat increased. It is probable therefore that the rapid loss in weight observed in pelagic animals kept in captivity is largely a pathological process, due to the partial disintegration of the tissues and loss of unoxidised compounds, whilst the animals are still alive; and that these unoxidised compounds, on standing in the water, undergo slow oxidation.

It may be remembered that it was shown that the respiratory activity of some pelagic animals, notably *Beroe*, *Cestus* and *Salpa pinnata*, increases considerably on successive days, though the condition of the animals at the same time obviously deteriorates. It may be thought that this apparent increase in respiration is due to the determinations being over corrected for the daily loss in weight of the animals. Thus we have seen that it is probable that the loss in weight is partly due to shrinkage of the tissues of the animal, so that though the actual weight of an animal may have considerably diminished, yet nearly as much proteid tissue may still be present, and be respiring as actively as before. It is in fact possible that some error may arise in this way, but it is only a very small amount. Thus in about a quarter of the observations made, it was not necessary to apply any correction at all for the loss in weight; in another quarter the correction so applied was less than 5 %; and only in one quarter of them was it more than 20 %. Now the increase observed in the respiration of *Beroe*, *Cestus* and *Salpa pinnata*, was 20 to 100 % per day, on successive days, or so great that not a tithe of it could be accountable to this source of error. We are now in a position to attempt an explanation of this remarkable phenomenon of the increase in the respiratory activity. The results just discussed have led us to conclude that pelagic animals kept in captivity get rid of a large part of their tissue substance in the form of unoxidised compounds. Now the worse the condition of an animal, and the longer it be kept, the greater is the tendency of the water in the respiration chamber to go putrid, especially if it be kept a few hours, or if the experiment itself be unduly prolonged. Probably therefore the compounds given off by the animal are in a more easily oxidisable and putrescible condition, the worse the condition of the animal. Thus though the actual respiratory activity of an animal may decrease considerably on successive days, yet the apparent respiration may be increased, as more of the oxidisable compounds, which are given

off equally during the whole course of the experiments, undergo oxidation in the water itself.

The Contractile Rhythm of Medusæ.

When making determinations of the respiratory exchange of the Medusa *Rhizostoma*, the contractile rhythm was noted from time to time, so that it is possible to examine and compare the effects produced by such conditions as the temperature of the water on the rhythm and the respiration at the same time. *Rhizostoma* is very suitable for these observations, as the animals generally continue their swimming movements with little or no intermittence for hours together. Romanes, in his research on the Locomotor System of Medusæ[1], made a few observations on the effect of temperature on the contractile rhythm of *Aurelia*, but he did not attempt to work out the relation between the two at all exactly. In studying the effect of temperature on the respiratory activity of *Rhizostoma*, it was found that on an average, if the respiration at 16° be taken as unity, that at 10° is ·52, and that at 24°, 1·93. The effect on the contractile rhythm is not so great, as in three experiments, the contractile rhythm at 10° was ·79, ·55 and ·76, or on an average ·70, and that at 24° 1·53, 1·55 and 1·32, or on an average 1·47, that at 16° being taken as unity as before. Also it was found that the respiratory activity of *Rhizostoma* increased at a greater rate than the temperature, or in geometrical proportion. The contractile rhythm on the other hand was found to increase regularly with the

Weight of Medusa = 93·0 grm.		Weight of Medusa = 150·0 grm.		Weight of Medusa = 69·6 grm.		
Temperature	Contractions per minute	Temperature	Contractions per minute	Days kept	Temperature	Contractions per minute
10·6°	43·5	9.7°	43	1	15·4°	61
10·8	44	10·8	44·5	10	13·6	48
16·1	55	12·8	49	11	12·9	48
17·0	57	18·5	61	12	11.7	44
19·0	64	20·0	66·5	13	11·9	43·5
19·9	65·5	20·7	73	15	11·3	43·5
20·5	67·5	22·5	79	16	10·6	41
23·0	69·5	23·5	84	19	9·3	28
23·5	73·5	24·0	84	20	8·8	31
		24·8	84·5	22	10·2	29
		25·4	85			
		26·2	86			
		26·5	85·5			
		27·5	79			

[1] *Phil. Trans.* 659. 1877.

temperature, or in arithmetical proportion, in four of the experiments made, and in geometrical proportion in the other two. In the table are given some of the figures on which these statements are based.

In the Medusa of which the data are given on the left side of the table, the rhythm increased regularly with the temperature; in that in the middle of the table, in geometrical proportion. The data given in this latter case also illustrate another point. Thus Romanes found that if a Medusa were put into sufficiently warm water, the rhythm was actually decreased. He did not however determine the temperature at which this unfavourable influence began to be exerted. In the present instance, the rhythm was found to increase normally with the temperature up to 23·5°. From this point till up to 26·5° the rhythm remained nearly constant, and then began to decrease again.

We have seen that the respiratory activity of *Rhizostoma* gradually diminishes on keeping the animals in captivity. The contractile rhythm follows a similar rule. To the right side of the table is given the contractile rhythm of a Medusa which was kept for over three weeks. During this time, the rhythm dwindled down from 61 contractions per minute to only 29, whilst the animal diminished some 80 % in weight. Romanes found that as a rule the rhythm of a Medusa bears an inverse proportion to the size of the individual. This conclusion was found to hold more or less generally in the case of *Rhizostoma* also, so the decrease in the rhythm in the above instance is even more marked than appears from the figures. A similar decrease was observed in other cases, in which the animal was kept for some time.

Romanes found that Medusæ were very sensitive to deficient aeration, such as takes place when they are confined in a small volume of water for a few hours. A similar conclusion was arrived at in the case of *Rhizostoma*. With some individuals the rhythm is only slightly affected, but in one instance it was diminished by about 20 % at the end of an experiment, in which only about half of the oxygen had been absorbed from the water. Two other experiments made on the same animal gave almost the same results. As the rhythm may be considerably affected by the gradual deoxygenation of the water, it might be thought that the respiratory activity is influenced to a similar degree. This is probably not the case, as we have seen that on keeping a medusa in captivity, its rhythm dwindles down very rapidly; and in fact, many individuals after a few days almost entirely cease their swimming movements, though they may remain alive for a much

longer period. The respiratory activity doubtless diminishes too, but not to so great an extent.

Summary.

The following are the chief conclusions arrived at in this paper:

The respiratory activity of the lower marine animals, such as the pelagic Cœlenterata, Tunicata, and Mollusca, is very small compared with that of the higher animals, such as the teleost fish. Thus that of the Tunicate *Salpa tilesii* is 60 times less, and that of the Ctenophore *Cestus* 44 times less than that of the Teleostean *Serranus*. *Amphioxus* has a value about a third as great. On the other hand the respiration of the Protozoon *Collozoum* appears to be about two-thirds as great as that of *Serranus*.

The respiratory activity of different animals is affected very differently by temperature. As a rule, the less differentiated and the more lowly the animal, the greater is the effect produced. Thus the 'temperature increment,' or relation of the respiratory activity at 24° C., to that at 10° C., is 4·9 for the Ctenophores *Beroe* and *Cestus*, 4·4 for the Tunicate *Salpæ*, and 3·0 for the Medusæ *Carmarina* and *Rhizostoma*. For the Molluscs *Pterotrachea*, *Octopus* and *Tethys* it is 2·6, and for the Vertebrates *Amphioxus*, *Heliases* and *Serranus* only 2·4.

The respiratory activity is very largely influenced by the size of the animal. Thus the respiration of a Rhizostoma weighing 62 grams was nearly three times greater than that of one weighing 107 grams. In the case of *Beroe*, whilst the extreme weights of the animals varied as 1 to 7·4, the respiration varied as 1·85 to 1. In *Cestus*, the weight varied as 1 to 2·9, and the respiration as 2 to 1. In fact there was no valid exception to the general rule that the respiratory activity of an animal varies inversely as its size.

On keeping animals in captivity, the respiratory activity of certain of them, as of the Medusæ, Mollusca and Vertebrata examined, either kept constant or slowly diminished on successive days, whilst that of others of them, such as *Beroe*, *Cestus* and the *Salpæ*, very rapidly *increased* from day to day, although the condition of the animals obviously deteriorated.

In pelagic animals, the respiratory quotients as a rule increase on successive days, but in the Vertebrata examined they remained constant. Most of the quotients are somewhat higher than those usually observed in warm-blooded animals. On asphyxiation of both the lower and

higher marine animals, quotients considerably greater than unity were obtained, a quotient of 4·45 being obtained in one instance with *Amphioxus*. The quotients of the animals after asphyxiation were also considerably greater than unity. The respiration of the transparent pelagic animals, after asphyxiation, appears as a rule to be even greater than in the living animals. This is probably due to the rapid onset of the processes of decomposition. In teleost fish however it is not a tenth as great.

The transparent pelagic animals contain a very small amount of solid organic matter in their tissues. Thus *Cestus* and *Salpa pinnata* contain respectively only ·24 and ·26 %. The other transparent animals experimented on contained ·4 to ·6 %. *Amphioxus* contains 12·8 %, and the fish *Heliases* 22·3 %. Calculated on the amount of solid constituents present, the respiratory activity of the lower marine animals is enormous, it being in most instances greater than that in man. As a general rule, for animals of the same class, it appears that the respiratory activity depends on the mass of the animal, and on the amount of solid constituents in its tissues.

On being kept in captivity, pelagic animals diminish very rapidly in size. *Rhizostoma* on an average decreases 8 % per day, *Beroe* and *Carmarina* 15 % per day, and the Mollusc *Pterotrachea* 30 % per day. This is four to ten times faster than one would expect from the respiratory exchange determinations, so much of the tissue substance must pass away unoxidised.

The contractile rhythm of the Medusa *Rhizostoma* is not affected by temperature to so great an extent as the respiratory activity. The rhythm rapidly decreases on keeping the animals in captivity.

In conclusion, I wish to express my thanks to the authorities at the Zoological Station at Naples, especially to Dr Schönlein, for the valuable suggestions and assistance they were always ready to afford me in the progress of this work. Also to Prof. Burdon-Sanderson my warmest thanks are due, for the encouragement and advice he has always been willing to offer me.

Appendix A.

The Composition of the Gases in the Sea Water.

In the table is given the daily composition of the gases in the water used in the respiration experiments. These values are calculated out as the number of cubic centimetres of gas at 0° C. and 760 mm. dry

pressure, present in a kilogram of water. This water was taken fresh every day from the large tank in the Aquarium. As the circulation in this water was not very great, the top layers were rather more oxygenated than those deeper down. Accordingly, when filling the bottle, it was plunged down nearly up to its neck in the water, and was held in the same position till it was filled by the water rushing in at the orifice at the bottom. This water was of course constantly undergoing oxygenation at the surface when in the bottle. In most days' experiments however less than half of the water was used up, for the vessel had a capacity of 16·5 litres, and so there was not time for the composition of the water used in the experiments to become affected enough to render a correction necessary. On many days, the water was analysed twice, at an interval of several hours, in order to determine how far the composition of the gases was affected by this diffusion at the surface, and in order to obtain greater exactness in the respiratory exchange determinations. On an average of thirty-six occasions, in which the analyses were made at an interval of not more than eight hours, the oxygen was found to be ·01 c.c. per kilogram greater in the second analysis, and the carbonic acid ·08 c.c. smaller. This difference in the carbonic acid is probably too large, as there are a few very high values, which considerably raise the average. On two occasions, when one analysis was made twenty-four hours after the other, the difference in the composition of the gases is considerable; it being ·59 c.c. greater for the oxygen, and ·75 c.c. less for the carbonic acid. In the analyses made at an interval of eight hours or less, the average difference in the oxygen determinations is ·09 c.c., and in the carbonic acid, ·27 c.c. As only about 300 grams of water were analysed at a time, the actual mean error of observation is only ·03 c.c. for the oxygen, and ·08 c.c. for the carbonic acid. These numbers are sufficient to show the great accuracy of the method. It may here be mentioned that the method seems very well adapted for the ordinary analysis of the gases of sea water. In the methods used by Dittmar and by Petterson and Eckman, two analyses are necessary; one for the determination of the oxygen and nitrogen, and the other for the determination of the carbonic acid. In the present method however a single analysis is sufficient to determine all three gases, with an accuracy equal to or greater than that obtained in these other methods.

It has been mentioned that in order to drive off all traces of gas from the evacuated boiling out flask, before the introduction of the water to be analysed, it is necessary to put a jar of warm water under

the flask, and boil the dilute sulphuric acid in it. In about the first fifth or sixth of the experiments made, this procedure was not adopted, and hence the oxygen and nitrogen determinations were too high. As however the gas driven off by the additional warming was found to have the composition of air, and as the volume of nitrogen present in the water remains practically constant, at any rate in all experiments made on the same day, it is possible to correct the oxygen determinations for this error. The water on an average contains about 12 c.c. of nitrogen per kilogram, so when in these analyses the gas was found to contain for instance 15 c.c. per kilogram, then $\frac{3}{15}$ of 3 c.c., or ·08 c.c., would be subtracted from the oxygen value. Even if the water actually contained 11 c.c. or 13 c.c. of nitrogen, no additional error would be introduced, as in order to determine the oxygen absorbed by the animal, it is only necessary to know the decrease in the oxygen present in the water, and not the absolute amount present. In the table are given the oxygen values corrected on the supposition that the water contained 12 c.c. of nitrogen per kilogram. They are thus directly comparable with the subsequent determinations, in which no correction had to be applied.

Petterson and Eckman, in their method of boiling off the carbonic acid in only a partial vacuum, found that in order to drive off the last traces of the gas, it was necessary to put iron wire in the acidified water. The bubbles of hydrogen thereby evolved seemed to assist the expulsion of the carbonic acid. In the present method, where the gases are boiled off in nearly a complete vacuum, the addition of iron wire is not necessary. Thus in the five experiments in the table marked with an asterisk, iron wire was added, the volume of carbonic acid evolved being + ·31, − ·10, ± ·00, + ·21, and − ·59 c.c., or on an average, − ·03 c.c. less than in analyses of the same water in which no iron wire was added. The apparent volume of nitrogen found in these determinations was of course larger than usual, as it contained in addition the hydrogen evolved by the action of the acid on the iron.

With regard to the analyses as a whole, we see that the carbonic acid varies between the limits of 65·68 to 74·29 c.c. per kilogram, and the oxygen from 3·28 to 5·74 c.c. per kilogram. The composition of the gases does not, as a rule, vary much from day to day, but the differences, such as they are, are too great for it to be possible to make analyses at less frequent intervals. On two occasions, viz. Jan. 28th and Feb. 19th, there are more considerable variations in the composition. These are due to large quantities of fresh sea water being pumped into the

circulatory tank system of the Aquarium. Thus on Feb. 19th the carbonic acid fell suddenly from 72·96 c.c. to 66·85 c.c. per kilogram, whilst the oxygen rose from 4·67 c.c. to 5·23 c.c. The proportion of oxygen present appears to be largely affected by the temperature. Thus in November, when the temperature was about 15° C., the water contained about 3·5 c.c. of oxygen per kilogram; in December and January, at a temperature of 11° C., about 4·2 c.c.; and in February, at a temperature of 9° C., about 4·7 c.c. This is due, not so much to the lesser solubility of the oxygen at the higher temperatures, for the water never contained its full proportion of oxygen, but probably to the greater respiratory activity and consequent absorption of oxygen by the animals in the Aquarium. In the experiments made from Jan. 8th to Jan. 14th, the water used was taken from a smaller tank, which held about 250 gallons of water. Though from the exposure of a relatively greater surface to the air, the water in this tank contained more oxygen and less carbonic acid than the water in the large tank, yet it was not possible to continue using it, as the composition of the gases varied so greatly in the water taken from different parts and strata in the tank.

The amount of nitrogen found in the water corresponds very closely with that found by Dittmar in the sea water shaken with air to saturation. In the following table are given the volumes of oxygen and nitrogen absorbed by one kilogram of water of the Specific Gravity 1·03044, as calculated from Dittmar's figures.

Temperature	Oxygen	Nitrogen	Nitrogen found
8°	6·56	12·64	
9	6·41	12·38	12·08
10	6·27	12·13	11·94
11	6·14	11·90	11·74
12	6·02	11·66	11·76
14	5·78	11·24	
16	5·56	10·84	

The values given in the last column of the table are the means of all the determinations made, in which the temperature of the water varied from 8·5° to 9·4°, 9.5° to 10·4°, 10·5° to 11·4°, and 11·5° to 12·0°. As the temperature of the tank water varied frequently and rather rapidly, and the equilibrium between the temperature and the nitrogen dissolved in the water is only reached very slowly, we see that at the lower temperatures the amount of nitrogen absorbed is not quite so great as the theoretical, whilst at the highest temperature it is somewhat greater.

The carbonic acid in the sea water was found to be on an average about 70 c.c. per kilogram. This is a very high amount, compared with the carbonic acid in the open sea water. Thus two analyses of a specimen of water taken about a mile from the shore in the Bay of Naples gave the following amounts of gases per kilogram:

Carbonic acid {46·88 c.c. / 46·91 c.c.} Oxygen {5·81 c.c. / 5·75 c.c.} Nitrogen {12·90 c.c. / 12·88 c.c.}

However the carbonic acid does not seem to have a very adverse influence upon the life of the fish kept in it, except as regards pelagic animals. This is probably because most of it is in loose combination with the salts in the water, and so the actual tension of the gas is very small. The amount which sea water can absorb, when quite saturated with the gas, is very large. Thus a specimen of water through which a stream of carbonic acid was passed for an hour, was found to contain, per kilogram, 218·86 c.c. of carbonic acid, 4·58 c.c. of oxygen, and 10·21 c.c. of nitrogen. The low tension of the gas in the water is also shown by an analysis of a specimen of water through which a rapid stream of air was allowed to bubble for four hours. The composition of the gases was changed thus:

From carbonic acid, 72·03 c.c., oxygen, 4·27 c.c., nitrogen, 11·92 c.c.,
to carbonic acid, 64·61 c.c., oxygen, 5·32 c.c., nitrogen, 10·41 c.c.

Thus the carbonic acid was only diminished by 9 %.

It was mentioned that in transferring the water from the animal chamber to the flask for analysis, it remained exposed for three or four minutes to the air. During this time the composition of the gases in the water would be slightly altered, by diffusion to and from the atmosphere. The error thus arising is however so slight as to be negligeable. In the following table are shown the changes taking place in the composition of the gases in various specimens of water, which were exposed to diffusion from the air under circumstances similar to those occurring in normal respiration experiments. After

Composition of gases before exposure		Composition of gases after exposure		Time of exposure in hours	Change in the	
Carbonic acid	Oxygen	Carbonic acid	Oxygen		Carbonic acid	Oxygen
71·36 c.c.	3·46 c.c.	70·88 c.c.	3·77 c.c.	2	− ·48 c.c.	+ ·31 c.c.
70·18	4·24	69·15	4·30	1½	− 1·03	+ ·06
75·12	2·06	75·08	1·98	½	− ·04	− ·08
77·20	·37	76·84	1·24	1½	− ·36	+ ·87
68·22	·32	68·30	·70	2	+ ·08	+ ·38

the water had been allowed to stand for periods varying from half-an-hour to two hours, it was stirred round gently a few times, so that the more oxygenated layers at the top of the jar might be mixed with the less oxygenated at the bottom, and was then analysed.

On an average, the water was allowed to stand for an hour and a half, and as a mean of five experiments, the proportion of carbonic acid was found to have decreased by ·37 c.c., whilst the oxygen had increased by ·31 c.c. As the water was not nearly saturated with oxygen at the end of the experiments, it would have been absorbing it at about the same rate during the whole time; and hence on an average in four minutes only ·014 c.c. would be absorbed. In the same time only ·016 c.c. of carbonic acid would have diffused away. Now as the determinations of the gases in the normal water would be subjected to this source of error as well as those of the gases in the water from the respiration chamber, the determinations of the actual respiratory exchange would practically be not affected at all. As the above analyses show, the oxygen is absorbed more rapidly when less is originally present in the water, and so in the analyses of the normal water the oxygen value would not be quite so much affected as in the analyses of the respiration water, but the difference in the absorption in the two cases would be inappreciable.

The Petterson's Apparatus used for the analysis of the gases proved to be very accurate and easily workable, but one important correction in the results was found to be necessary. Thus the tube connecting the potash absorption bulb with the measuring tube was found to have an internal volume of ·50 c.c., though if it had been millimetre tubing, as was supposed, its volume would have been only ·10 c.c. After the absorption of the carbonic acid from the mixed gas, and when the gas is driven over into the pyrogallate bulb for the absorption of the oxygen, there is always this volume of ·50 c.c. which never reaches the pyrogallate. If now the gas analysed contains almost the same volume of oxygen as in the previous analysis made with the apparatus, no error is introduced. If however it contains either more or less oxygen than in the previous determination, then a small additional amount of oxygen is left in this tube, or is taken away from it, and an error is introduced. In extreme cases an error of ·12 c.c. can thus be brought about in the oxygen determination, though the average error is not so large as this. Nevertheless it would be sufficient to increase most of the respiratory quotients found by about 10 %, unless a correction were made for it.

Date of Experiment	Number of hours' interval	Temperature of water	Carbonic acid per kilogram	Difference between two carbonic acid determinations	Oxygen per kilogram	Difference between two oxygen determinations	Nitrogen per kilogram
					3·47 c.c.		12.00 c.c.
Nov. 16		17·7°	68·72 c.c.		3·49		12·00
17		17·7	69·09		3·58		12·00
19		17·0	69·47		3·58		12·00
22		16·5	69·96		4·42		12·00
23		15·7	69·30		3·87		12·00
24		15·2	69·34		3·79		12·00
27		13·9	69·26		3·53		12·00
28		13·9	70·85		3·55		12·00
29		14·2	70·14		3·46	− ·09	12·00
29	7	14·2	69·75	− ·39	3·31		12·00
30		14·2	70·80		3·46	+ ·15	12·00
30	5	14·2	70·72	− ·08	3·28		12·00
Dec. 1		14·0	71·28		3·70		12·00
3		12·8	70·22		4·16		12·00
4		12·9	70·67		3·94	− ·22	12·00
4		12·9	70·67	± ·00	4·14		12·00
5		12·9	71·13		3·92	− ·22	12·00
5		12·9	71·31	+ ·18	3·50		12·00
6		13·0	71·36		3·88		12·00
7		13·3	71·00		3·62		12·00
8		13·5	72·22		3·80		12·00
10		13·0	72·60		3·87		12·00
11		12·7	71·89		4·05		12·00
12		12·4	71·59		3·95		12·00
13		11·9	71·77		3·98	+ ·03	12·00
13	5	11·9	70·96	− ·81	4·11		12·00
14		11·7	71·18		4·22	+ ·11	12·00
14	4½	11·7	71·54	+ ·36	4·33		12·00
15		11·8	69·18		4·16	− ·17	12·00
15	6	11·8	69·13	− ·05	4·24		12·00
17		11·7	69·77		4·31	+ ·07	12·00
17	7	11·7	69·83	+ ·06	4·17		11·96
18		11·2	69·72		4·24		12·00
19		10·9	70·46		4·18	± ·00	11·78
19	4	10·9	69·90	− ·56	4·22		11·89
20		11·2	70·30		4·08	− ·14	11·79
20	6	11·2	69·88	− ·42	4·24		11·69
21		11·3	70·13		4·19		11·63
22		11·0	69·86		4·53		11·41
24		10·6	70·83		5·03		12·20
26		10·3	69·86		4·96		12·48
27		10·0	70·06		5·04	+ ·08	12·44
27	4	10·0	70·21	+ ·15	4·79		12·82
28		9·9	70·21		4·80		12·00
29		9·8	71·35		4·78	− ·06	12·15
29	1	9·8	71·10	− ·25	4·90		12·18
31		9·8	70·52		4·80		12·26
Jan. 1		9·7	70·56		4·64		12·32
2		9·4	70·75		4·81	+ ·17	12·24
2	0	9·4	70·13	− ·62	4·80		12·23
4		9·0	68·60		4·88	+ ·08	12·19
4	4	9·0	69·33	+ ·73	4·91		12·33
5		9·1	69·45		4·93		12·50
7		9·0	68·94		5·01	+ ·08	12·32
7	6½	9·0	68·48	− ·46	5·54		11·26
8		9·6	68·05		5·58	+ ·04	11·53
9	22	9·6	68·44	+ ·39	5·64		11·43
10		9·8	68·40		5·46	− ·18	11·19
11	18	9·8	68·23	− ·17	5·74		11·38
14		10·9	69·07				

Jan. 15		10·6	71·11		4·90		11·97
16		10·6	71·21		4·74		12·13
16	8	10·6	71·32	+ ·11	4·77	+ ·03	12·34
17		11·2	71·69		4·39		12·13
17	5	11·2	71·60	− ·09	4·32	− ·07	12·17
18		11·5	72·03		4·27		11·92
19		11·5	72·14		4·06		11·91
19	1	11·5	72·44	+ ·30	4·06	± ·00	11·81
21		11·6	72·30		4·12		12·19
21	1	11·6	72·61*	+ ·31	4·12	± ·00	12·50
22		11·8	73·06		4·13		12·03
22	7	11·8	72·96*	− ·10	4·12	− ·01	12·91
23		11·9	73·36		4·10		12·03
23	4½	11·9	73·36*	± ·00	4·14	+ ·04	13·78
24		12·0	73·60		3·85		11·98
24	8	12·0	73·72	+ ·12	3·69	− ·16	11·85
25		11·6	73·86		3·82		12·06
25	7	11·6	74·07*	+ ·21	3·74	− ·08	17·67
26	24	11·6	72·94	− ·92	4·46	+ ·64	11·42
28		11·1	74·29		3·98		11·65
28	1	11·1	73·70*	− ·59	3·91	− ·07	20·13
29		11·1	70·29		4·41		11·63
29	6	11·1	69·57	− ·72	4·50	+ ·09	11·55
30		10·5	70·73		4·18		11·58
31		10·3	70·78		4·34		11·88
Feb. 1		9·8	71·15		4·30		11·90
1	5	9·8	70·83	− ·32	4·41	+ ·11	11·88
2		9·5	71·12		4·65		12·08
2	6	9·5	71·45	+ ·33	4·65	± ·00	12·06
4		9·6	71·35		4·56		12·03
4	5	9·6	71·37	+ ·02	4·71	+ ·15	11·90
5		9·5	71·95		4·48		11·92
6		9·5	71·48		4·47		11·85
6	6	9·5	71·57	+ ·09	4·40	− ·07	11·86
7		10·0	71·89		4·44		11·99
7	5	10·0	71·40	− ·49	4·55	+ ·11	11·86
8		10·5	72·32		4·27		11·87
9		10·8	72·36		4·49		11·94
11		10·9	72·57		4·62		11·74
11	7	10.9	72·67	+ ·10	4·56	− ·06	11·77
12		11·5	72·58		4·79		11·55
13		11·6	72·39		4·43		11·33
14	24	11·6	71·81	− ·58	4·98	+ ·55	10·98
15		10·8	72·72		4·40		11·23
15	6	10·8	72·73	+ ·01	4·43	+ ·03	11·21
16		10·9	74·12		4·23		11·36
18		8·8	72·96		4·67		12·24
19		8·9	66·85		5·23		11·60
19	2	8·9	67·12	+ ·27	5·33	+ ·10	11·61
20		8·9	65·68		5·13		11·64
21		8·6	66·51		5·04		11·85
22		8·6	67·25		4·86		11·94
22	7	8·6	67·21	− ·04	5·05	+ ·19	11·91
25		8·5	68·21		4·73		11·97
25	2	8·5	68·02	− ·19	4·85	+ ·12	12·01
26		9·0	68·52		4·61		11·95
27		9·3	69·31		4·53		11·45
28		9·6	69·09		4·40		11·68
28	4	9·6	68·76	− ·33	4·35	− ·05	11·61
Mar. 1		9·3	69·35		4·76		11·81

Appendix B.

The Salinity of the Sea Water.

As has been already mentioned, it was necessary from time to time to determine the amount of salt in the water used in the experiments, in order to determine the total solid organic constituents in the animal tissues. The saline residue obtained on evaporation on a water bath was dried in an air bath at 100° C. for forty to fifty hours. It was afterwards dried again for twelve hours at 120° C. to 140° C., and weighed, as a control on the determination at 100° C. Even at 140° C. not all the moisture is driven off, a direct determination of the salt in sea water being in fact impossible: for at a temperature high enough to drive off all the water, some of the magnesium chloride is decomposed. In a few cases the Specific Gravity of the water was taken by means of a Specific Gravity bottle. It will be seen that the values are very high, as normal sea water has on an average a Specific Gravity of 1·0267.

The salinity of the tank water in the Aquarium underwent considerable variations when large quantities of fresh sea water were pumped into it. As the analyses of the gases in the water show, this only occurred on two occasions during the course of the experiments, namely, on Jan. 28th and on Feb. 19th. From the date of the commencement of the experiments up to Jan. 27th, the salinity of the water underwent a steady rise. As determinations of the salinity were only made at intervals of a few days, and it was frequently necessary to know the salinity on intermediate days, all the values found were plotted out in the form of a curve, and the salinity on the other days determined. The values used for the salinity before Dec. 21st are only calculated ones, as no actual determinations were made before this date. Between Jan. 28th and Feb. 19th the salinity appeared to keep practically constant.

Date	Grams of salt per kilogram	Sp. G. at $\frac{15°}{15°}$
Dec. 21	42·986	
Dec. 28	43·176	
Jan. 4	43·207	
Jan. 16	43·645	
Jan. 24	44·008	
Jan. 25	43·939	
Jan. 30	43·461	1·0310
Feb. 5	43·545	1·0306
Feb. 13	43·530	1·0304
Feb. 20	43·275	1·0299
Feb. 28	43·175	1·0303

THE INNERVATION OF THE PELVIC AND ADJOINING VISCERA.

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PART II. THE BLADDER.

Previous Observations on Efferent Vesical Fibres[1].

As we shall deal almost exclusively with the efferent nerves of the bladder, we omit reference to previous work on its rhythmic contraction, its internal pressure, its afferent nerves[2], and restrict our account of the earlier observations to those which relate to the origin from the spinal cord of the efferent bladder fibres and the course these fibres take.

First as to the upper set of nerves. Gianuzzi, experimenting after the manner of Budge, found that contraction of the bladder in the dog was obtained by pricking the spinal cord at the level of the lower part of the 3rd lumbar vertebra, and that the contraction usually ceased on section of the nerves called later the hypogastric nerves; sometimes in order to stop the effect it was necessary to cut also strands said to run to the bladder from the upper sacral sympathetic ganglia. He concluded that motor fibres ran to the bladder from the lumbar spinal cord, traversing on their way the sympathetic chain and the inferior mesenteric ganglia. The argument is not quite satisfactory, since the absence

[1] Budge. *Virchow's Archiv*, xv. 115. 1858.
Gianuzzi. *Journ. de la Physiol.*, vi. 22. 1863.
Budge. *Zeitsch. f. rat. Med.*, xxi. 1 u. 174. 1864.
Budge. *Pflüger's Archiv*, vi. 306. 1872.
Sokownin. *Pflüger's Archiv*, viii. 600. 1874.
Sokownin. *Jahresberichte* (Hofmann u. Schwalbe), 1877. Abt. ii. p. 87.
H. Nussbaum. *Jahresberichte* (Hofmann u. Schwalbe), 1879. Abt. ii. p. 64.
Langley. *Journ. of Physiol.*, xii. (*Proc. Physiol. Soc.* Dec. 1890).
Navrocki u. Skabitschewsky. *Pflüger's Archiv*, xlviii. 335. 1891.
Sherrington. *Journ. of Physiol.*, xiii. 678. 1892.

[2] We may mention that so far as our experiments go the origin and course of the afferent fibres is the same as that of the efferent fibres of the bladder.

of effect might be due to the progressive decrease in irritability which takes place in the spinal cord after exposure; this explanation seems to be the one adopted by Budge. Sokownin made observations of a similar nature to those of Gianuzzi but did not describe any fibres as passing from the lumbar cord to the bladder except by the hypogastric nerves. He advanced a step by showing that stimulation of the cut peripheral ends of the hypogastric nerves causes contraction of the bladder. H. Nussbaum confirmed Sokownin except that he found that some fibres continued on in the sympathetic chain. This was a return to Gianuzzi's view but with less definiteness as to the course taken by the fibres. Langley stimulated the sympathetic chain from above downwards, taking precautions against reflexes from the spinal cord; he found in the rabbit contraction of the bladder on stimulating the sympathetic from about the 2nd to the 6th lumbar ganglia; indicating that the motor fibres of the bladder arise from about the 1st to the 5th lumbar nerves. The branches from the sympathetic to the inferior mesenteric ganglia also gave contraction. Navrocki and Skabitschewsky stimulated in the cat the lumbar nerves in the vertebral canal and found contraction of the bladder from the 4th and 5th lumbar nerves. They found no effect after section of the hypogastric nerves, and no effect on stimulating the sympathetic a little above the sacral region. Sherrington, in the cat found contraction of the bladder on stimulating the 3rd lumbar nerve, as well as the 4th and 5th, and in two cases out of seven, he obtained a slight effect also on stimulating the 2nd lumbar nerve. In the monkey he obtained contraction from the 2nd, 3rd and 4th lumbar in eight cases, and from the 3rd, 4th and 5th in one case. It will be seen, that the points which require further elucidation with regard to the upper set of nerves are (1) whether two, three or more lumbar nerves have efferent fibres for the bladder, (2) how far these vary in different animals of the same species in correspondence with the anterior, median and posterior arrangement of the spinal nerves, (3) whether any of the fibres take a course other than that of the hypogastric nerves, and if so what exactly the course is.

Passing now to the lower set of nerves. Gianuzzi showed that fibres causing strong contraction of the bladder issue from the spinal cord in the sacral nerves running by the nerve now generally called the nervus erigens to the hypogastric (pelvic) plexus. He found that the nervus erigens arose in the dog from three sacral nerves. His statement that these nerves are the 3rd, 4th and 5th sacral we may dismiss as probably due to a desire to correlate the nerves of the dog with those of man.

Budge's concise remark in 1872 that in the dog, the efferent nerves to the bladder are contained in the 1st, 2nd and 3rd sacral nerves is the earliest we need be concerned with. H. Nussbaum found the same to be the case in the cat. He distinguished, however, between the 2nd and 3rd sacral nerves which caused strong contraction of the bladder, and the 1st sacral nerve, which caused a comparatively weak contraction. Langley obtained similar results to Nussbaum as regards the cat and dog; but found that in the rabbit the effective nerves were one segment lower in origin. Sherrington, also, found in the cat, the three sacral nerves to contain efferent fibres for the bladder, but in his experiments, the 2nd sacral nerve had the maximal effect, after this came sometimes the 1st and sometimes the 3rd sacral nerve. His results so far as they went, seemed to show that this variation was in correspondence respectively with an anterior and a posterior arrangement of the spinal nerves. In the monkey, in two experiments out of nine, the 3rd sacral nerve had no effect. Navrocki and Skabitschewsky found efferent fibres for the bladder of the cat in two nerves only, viz., in the 2nd and 3rd sacral nerves.

The Lumbar Nerve Fibres to the Bladder.

Function. The upper set of nerves cause contraction of the whole musculature of the bladder; but the contraction is strongest at the base of the bladder near the entrance of the ureter. These nerves have, in the cat at any rate, little if any effect on the urethra.

Stimulation of the nerves on one side causes contraction of both sides, in consequence of the decussation of fibres in the inferior mesenteric ganglia[1].

The theory that the lumbar nerves cause contraction of the circular coat and not of the longitudinal coat is untenable. It is hardly contested that histologically two separate coats do not exist—a point that has recently been insisted on by Griffiths[2]—for the bundles of longitudinal fibres are obviously continuous with the bundles of oblique and of circular fibres. As regards actual experiment it will be remembered that Gianuzzi described the contraction produced by stimulating the lumbar spinal cord as being of the same kind as, though weaker than, that produced by stimulating the sacral nerves. And both Langley and Griffiths have observed directly contraction of circular and longi-

[1] Langley and Anderson. This *Journal*, XVI. 423. 1894.

[2] Griffiths. *Journal Anat. and Physiol.*, XXV. 540. 1894; XXVI. 60 and 255. 1895.

tudinal muscles on stimulating either the hypogastric nerve or the pelvic nerve (nervus erigens).

We have stimulated sometimes the lumbar nerves in the spinal canal, sometimes the peripheral ends of the hypogastric nerves, and then investigated the contraction of the outer longitudinal bundles of the bladder in three ways.

(1) The muscular coat during its contraction is observed with a hand-lens. The shortening of the longitudinal as well as of the other muscular bundles is readily seen.

(2) One leg of the animal is held up by a clamp in such a way that the bladder—nearly emptied for the purpose—hangs downward, and the urine remaining in it slightly elongates it. On stimulation of the lumbar nerves or of the hypogastrics, the bladder undergoes very obvious shortening. This experiment should be made before exposure to air has caused the bladder to enter into tonic contraction.

(3) The bladder is cut into longitudinal strips. The strips shorten on stimulating the upper set of nerves in any part of their course; the edges of the strips at the same time curl over.

We may mention here that it can be shown in a similar manner that the pelvic nerve (nervus erigens) contains motor fibres for the circularly arranged as well as for the longitudinally arranged muscles of the bladder.

In view of these facts we have not thought it necessary to repeat the experiments of v. Zeissl[1], especially as they are obviously open to an interpretation other than that which he adopts. It can easily be seen, in the conditions of his experiments, that a longitudinal contraction of the basal portion of the bladder would tend to draw the urethra forward and so give the effects he obtained on stimulating the hypogastric nerve; and that a strong contraction, even if it involved the urethra, would tend to widen the urethral orifice, and so give the effects he obtained on stimulating the nervus erigens. Since v. Zeissl's experiments were made on male dogs, the results are complicated by the presence of the prostate gland, which in these animals is close to the bladder.

The contraction of the bladder produced by the lumbar nerves (or hypogastrics) is not strong enough to cause micturition; but this is not peculiar to the lumbar nerves, for one of the sacral nerves is often equally ineffective. Indeed in some cases one of the sacral nerves causes a weaker contraction than is caused by the 4th or 5th lumbar nerve.

[1] v. Zeissl. *Pflüger's Archiv*, LIII. 560. 1893.

Another matter, which is not so clear, is that of the presence of inhibitory fibres for the bladder in the upper set of nerves. The apparent presence of inhibitory fibres in the hypogastrics was noticed by one of us some time ago (L. *op. cit.* 1890). In the experiments indicating their presence, a tube was passed through the urethra into the bladder, and tied in the urethra, and the tube connected with a burette filled with salt solution. On stimulating the peripheral end of the hypogastrics, there was often a fall of the fluid in the burette after a slight transient rise. The same fact was observed by Griffiths about the same time, and in his recent Paper, he states, that in the dog the inhibitory effect is best seen after the bladder has been made to contract by stimulating the nervus erigens; the subsequent relaxation is quickened by stimulating the hypogastrics.

In a considerable number of experiments on the contraction of the bladder in which we used the graphic method, we obtained not infrequently, on stimulating the lumbar nerves or the hypogastrics a fall of the internal pressure of the bladder, considerably greater than the preliminary rise due to contraction. In one or two cases there was barely any rise before the fall of pressure.

On examining the bladder directly however, we could find no satisfactory evidence of the presence of inhibitory nerve fibres in the hypogastrics. A slight flaccidity follows the contraction brought about by the hypogastrics, but we never observed the flaccidity without the contraction, nor any considerable degree of flaccidity. Moreover we were not able to satisfy ourselves that the normal relaxation after sacral stimulation was quickened by stimulating the hypogastric nerves, and they certainly had no obvious inhibitory effect on the bladder when it had become contracted in consequence of exposure to the air. It seemed clear to us that if the hypogastrics contain inhibitory fibres, their action could only be slight, and that a special series of experiments would be necessary to determine definitely whether or no any such fibres exist. We have consequently not gone further into the matter.

The lumbar set of nerves have very little if any effect on the arteries of the bladder. Occasionally, the hypogastrics seemed to give slight constriction of the small arteries and slight pallor: but in most cases there is undoubtedly no effect. The small arteries do not contract and the pulsation in them is seen in undiminished extent. The result is the more striking since the small arteries of the intestine, of the uterus, and of the penis, on stimulation of the upper set of nerves take ordinarily but a few seconds to disappear completely from view.

Origin. In the following Table we give the results of our experiments as to the lumbar nerves which cause contraction of the bladder. The symbols S. M. etc. are used with the same meaning as in earlier Tables (cf. Part I. p. 84).

Course taken by the lumbar nerve fibres. In a considerable number of cases we have found no effect on the bladder from stimulation of the lumbar sympathetic or of the lumbar spinal nerves, after the hypogastric nerves have been cut. But sometimes a slight contraction is still obtained. In most cases the fibres which cause this contraction run to the pelvic plexus from the aortic plexus or from about the region of the 6th lumbar ganglion. For it is rare to obtain contraction of the bladder either in cat or rabbit, by stimulating the sympathetic at or below the 7th lumbar ganglion. Since however this does occur occasionally—and we give an instance of it in Protocol II.—we conclude that a few fibres for the bladder occasionally leave the sympathetic for the pelvic plexus as low down as the 1st sacral ganglion.

We have said[1] that in the cat, the hypogastric nerve commonly divides into two strands, the smaller and more dorsal we have called the accessory hypogastric. Sometimes the division takes place close to the inferior mesenteric ganglion; when this variation occurs, it is easy to determine that the larger strand, the hypogastric proper, supplies chiefly the bladder and the uterus (or vas deferens), and that the accessory hypogastric supplies chiefly the rectum and internal anal sphincter. In one or two cases the accessory hypogastric has had no effect at all on the bladder. An instance of this is given in the following Protocol of an experiment.

Protocol II.[2] *Cat. Stimulation of the pudic nerve, of the sympathetic trunk after section of the pudic, of the hypogastric and accessory hypogastric.* Chloroform then A. C. E. mixture by tracheal tube. Tie and cut pudic nerves on both sides.

Stimulate peripheral ends of left pudic nerves. Strong contraction of external anal sphincter and of elevator of scrotum; quick projection of penis, penis soon becomes bluer, then pale and retracts. Contraction of tunica dartos of scrotum.

Tie and cut on both sides the sympathetic chain below the 6th lumbar ganglion, isolate each up to the 7th ganglion. Tie and cut left hypogastric

[1] This *Journal*, XVI. 413. 1894.

[2] In this and in other cases, we select for detailed description an experiment which, in addition to showing the action of the nerves upon the particular viscus we are discussing at the moment, shows also the action of the nerves upon the viscera generally.

Origin of Upper Set of Nerves to the Bladder.

	Dog			Cat									Rabbit					
Exp.	8 11	1 3. 7[1]	4 5	59	21	65	27	63	26	64	20	56	27	37	9	23	32	31
I Lumbar	—	0	—	—	—	—	0	0	0	—	0	0	0	0	0	—	—	—
II ,,	M	S	—	—	?S	—	?S	0	0	0	0	0	S	0	0	—	—	—
III ,,	M	M	—	—	M	—	S	S	?0[2]	S	S	S	M	S.M	S	C	—	—
IV ,,	0	M	M	M	M	M	M	M	C	M	M	M	—	M	C	C	M	—
V ,,	—	0	0	0	0	0	S	S.M	C	S	M	M	—	M	C	C	M	M
VI ,,	—	0	0	0	0	—	—	—	0	0	—	0	—	0	—	0	—	0
Lumbo-sacral Nerves	Ant.	—	4 M to P 5 Post.	Ant.		Med.			Post. (*a*)		Post. (*b*)		Ant.	A to M	M	M to P	P	

M indicates a greater contraction than M.

C ,, that a contraction was observed, but its strength not compared with that from other nerves.

In all of these Exps., except Nos. 20 and 21, the bladder was directly observed. In these two a tracing was taken.

[1] No. 7. Plexus posterior; there were 13 thoracic but only 6 lumbar vertebræ.

[2] The anterior ramus to the inferior mesenteric ganglion was cut.

nerve. On the right side the hypogastric consists of two distinct nerves, the smaller being the 'accessory' hypogastric, tie and cut these separately.

Stim. left sympathetic. No effect seen on penis or scrotum. Strong erection of hairs of tail; this occurred with all later stimulations of the sympathetics. Some contraction of bladder.

Stim. right sympathetic. Effects like those of left, but less contraction of bladder.

Stim. both hypogastrics. Trace of movement of penis; no effect on scrotum, good contraction of vasa deferentia, moderate contraction of bladder.

Stim. accessory hypogastric. No effect on bladder, slight longitudinal and circular contraction at junction of colon and rectum.

Stim. right hypogastric. Contraction of bladder, no certain effect on colon or rectum.

Tie and cut the left nervus erigens, with its accompanying blood vessels, close to the pelvic plexus.

Stim. left sympathetic. No effect seen on bladder.

Stim. right sympathetic. Apparently slight contraction of bladder.

Cut through prepuce, free the penis from surrounding tissue up to limb muscles.

Stim. the medio-posterior branch of the left nervus erigens. Slow enlargement and protrusion of the penis.

Stim. left sympathetic. Slight shrinking of the penis.

Stim. nervus erigens to obtain erection, then

Stim. right sympathetic. Slight shrinking of the penis.

Stim. hypogastrics. Slight further shrinking of the penis.

The Sacral Nerve Fibres to the Bladder.

There are in the dog and rabbit generally three nerves in the sacro-coccygeal region which contain motor fibres for the bladder. In the cat there are three such nerves if the lumbo-sacral plexus is anterior in arrangement, otherwise two only. But so far as our experiments go, two nerves only, are capable in normal conditions of causing micturition. In the cat these are, in nearly all cases, the 2nd and 3rd sacral nerves, and in the rabbit the 3rd and 4th sacral nerves. The contraction produced by the remaining nerve, then, is not sufficiently powerful to cause expulsion of the urine.

We have generally taken note in our experiments whether micturition was produced or not. In giving a brief statement of the results in the cat, we will omit the experiments in which, in addition, the bladder was observed directly. The experiments were 15 in number. The 7th lumbar and 1st coccygeal had no effect in any case. In two

cases (Nos. 6 and 45, Nerves anterior) the 1st and 2nd sacral caused some micturition, the 2nd sacral having the stronger action: in the thirteen other cases, micturition was produced by the 2nd and 3rd sacral nerves only. The arrangement of the lumbo-sacral nerves in these was; anterior in two (Nos. 2, 5), anterior to median in one (No. 32), median in four (Nos. 13, 37, 40, 58), median to posterior in two (Nos. 46, 52), moderately posterior in two (Nos. 39, 44), very posterior in two (Nos. 17, 29).

Although two nerves cause micturition, there is always a more or less marked difference in their action. The anterior of the two nerves, as a rule, produces micturition only after the cessation of the stimulus to the nerve, or after the stimulus has been continued for some time; the posterior one, on the other hand, causes micturition at once, allowing for the ordinary latent period of unstriated muscle. The difference in the effect is due to the fact that the anterior nerve contains efferent fibres for the striated muscles of the urethra and generative organs, whilst the posterior does not. On giving curari, or on cutting the pudic nerves, the difference is no longer seen. And in such case micturition *may* be produced, though it usually is not, by the remaining of the three nerves which contain efferent fibres for the bladder.

In the rabbit when there is much resistance, to the outflow of urine, strong contraction of the bladder drives urine into the seminal vesicles (uterus masculinus) or into the muscular upper portion of the vagina. This usually sets up rhythmic contraction of these organs. The seminal vesicles may drive the urine back again, though usually incompletely. Sometimes raising the lower part of the body by the tail compresses the urethra sufficiently to produce this result.

When the sacral nerves are repeatedly stimulated, and the bladder is not allowed to empty itself, it becomes less responsive, and thus a nerve which at the beginning of an experiment caused a slight contraction, may later produce none.

We have not been able to satisfy ourselves that the sacral nerves have any action on the vesical arteries. When they cause strong contraction there is great pallor of the bladder, but this may be merely a mechanical effect. When the contraction is greatly diminished by a large dose of atropin (cp. below p. 82) the sacral nerves have still no obvious vaso-motor action.

Unilateral action of the sacral nerves. Gianuzzi mentioned that the sacral nerves of one side caused contraction chiefly of the same side of the bladder, but he does not appear to have given any close attention

to the matter. According to Griffiths and to Sherrington the action of these nerves is strictly unilateral. We have made a few observations on the rabbit and cat and we do not find a strictly unilateral action. In our observations we cut through the bladder in the middle line, a variable distance from the tip towards the opening of the ureters. We then stimulated on one side one or more of the carefully isolated sacral nerves, or the pelvic nerve (nervus erigens) near its origin. In five cases out of six there was distinct, though in most cases slight, contraction in the part of the bladder on the opposite side which had been separated by the cut from its fellow portion: and this occurred whether the bladder on the same side was allowed free movement, or clamped at its tip in order as far as possible to prevent movement. If the bladder be not emptied, then as described by Griffiths, the half on the stimulated side contracts, whilst the opposite side is bulged out.

Origin. We give below, the experiments we have made upon the origin of the sacral nerve fibres to the bladder. It will be seen that our results as regards the cat explain most of the discrepances in earlier results; thus Nussbaum probably experimented with cats in which the arrangement of nerves was anterior, and Navrocki with cats in which the arrangement was posterior. It is true, that Sherrington describes the 1st sacral as giving sometimes a stronger contraction than the 3rd sacral; this we have not found, but it is perhaps possible that in his cases the nerves were arranged more anteriorly than in any of those marked 'Anterior' in our Table of Experiments.

Origin in Rabbit of Lower Set of Nerves to the Bladder.

Exp.	18	37	30	29	4/20	36	32	22	21/28	10	33/34	31
I Sacral	0	0	0	0	—	0	—	0	0	0	0	0
II ,,	G	S	0	?S	—	0	—	0	0	0	0	0
III ,,	G	G	G	G	G	G	G	G	G	G	G	M
IV ,,	S	G	—	S.M	G	G	G	G	G	G	G	G
I Coccygeal	0	0	—	0	?0	S	—	—	S	S	M	M
II ,,	—	—	—	—	—	—	—	—	0	—	0	0
	A	A to M	Med. to Ant. (about)		—	M to P	Posterior					

Origin in Cat and Dog of Lower Set of Nerves to the Bladder.

	Cat													Dog			
Exp.	19 24	57[1] 59	21[2] 22		60 63	62	16	49 61	53	54	50 55	64	56	6 2	4	5	7[3]
VII Lumbar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I Sacral	S.M	S	C	S	S	0	0	0	0	0	0	0	0	S.M	0	0	0
II ,,	G	G	C	G	G	G	G	G	G	G	G	G	G	G	G	G	G
III ,,	—	G	C	G	G	G	G	G	G	G	G	G	G	G	G	G	G
I Coccygeal	—	0	0	0	0	0	0	0	0	0	0	0	0	0	S	S	M
Arrangement of Nerves	Anterior			—	Median		—	Median to Posterior			P(*a*)		P(*b*)	6 Ant. 2 A to M	M to P	Post.	

[1] Cat had 14 thoracic nerves.

[2] Relative degree of contraction of I S. and III S. not noted.

[3] There were only 6 lumbar nerves.

Note on the action of some alkaloids. When a tracing of the internal pressure of the bladder is taken, it is seen that intravascular injection of a small quantity of nicotin causes, first a strong contraction, and subsequently a very considerable dilation. Curari usually causes a moderate primary contraction. The effect of atropin on the tone of the bladder we have not noticed; a moderate dose has very little action upon the effectiveness of the efferent nerves. A large dose weakens the contraction produced by nerve stimulation; thus in an experiment on a dog 150 mgrms. of atropin were injected into the jugular vein; then stimulation of the 1st sacral nerve—which previously had caused distinct though slight contraction—had no effect, strong contraction was however produced by stimulation of the 2nd and 3rd sacral nerves. A further injection of 100 mgrms. was made; the 2nd and 3rd sacral nerves still had an effect, though comparatively slight; the contraction also was transient, occurring at the beginning of the period of stimulation and rapidly disappearing. In the rabbit and cat 50 mgrms. is the maximum amount we have injected; the contraction of the bladder from nerve stimulation was weakened, but to the eye, not very greatly.

Summary.

The results which we have obtained as to the origin and course of the efferent fibres to the bladder are in large part simply confirmatory of facts which have been already shown by some one or other observer. (Gianuzzi, Budge, Sokownin, H. Nussbaum, Langley, Navrocki and Skabitschewsky, Sherrington.) But we have been able to give more definiteness to the account, by explaining the discrepancies which exist in the previous statements. The discrepancies are due, as a rule, to the experiments having been insufficient in number, so that the variations which occur in different individuals escaped notice.

The slight, and somewhat variable effect, which the lumbar nerves have on the bladder, makes it difficult to determine with any exactness their relative efficiency. And, in consequence, we have not always found such marked variations to exist in the lumbar innervation of the bladder according to the arrangement of the lumbar nerves, as we have found in other cases. But the details we have given on p. 77, afford we think some evidence of the following scheme of innervation.

	Dog		Cat			Rabbit	
	Anterior	Posterior	Anterior	Median	Posterior	Anterior	Posterior
II L.	Moderate	Slight	? Slight	0	0	Slight	0
III L.	Moderate	Moderate	Moderate	Slight	Slight	Moderate	Slight
IV L.	0	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
V L.	0	0	0	Sl. to Mod.	Moderate	Moderate	Moderate
VI L.	0	0	0	0	0	0	0

It will be noticed that the origin of the nerves to the bladder is more anterior in the dog than in the cat. This is probably connected with the greater development of nerves to the hind limb.

The variations in sacral nerve supply work out with greater certainty. They are

	Cat		Dog		Rabbit	
	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
VII L.	0	0	0	0	0	0
I S.	Sl. to Mod.	0	Sl. to Mod.	0	0	0
II S.	Good	Good[2]	Good	Good	Sl. to Mod.	0
III S.	Good	Good[2]	Good	Good	Good	Good
IV[1]	0	0	0	Sl. to Mod.	Mod. to Good	Good
V[1]	0	0	0	0	0	Sl. to Mod.

[1] We have, for simplicity, numbered these from the 1st sacral downwards; IV is the 1st coccygeal in the cat and dog; V is the 1st coccygeal in the rabbit.

[2] Sometimes one, sometimes the other appears to have the maximum effect.

We have omitted in this scheme the median arrangement of nerves; this is intermediate between the anterior and posterior, and the sacral innervation of the bladder inclines towards that occurring with an anterior or a posterior plexus, according as the plexus itself is on the anterior or posterior side of median.

It will be noticed that in the dog the 1st coccygeal nerve sends fibres to the bladder when the plexus is posterior, and that this is not the case in the cat. This is probably due, like the similar difference in the lumbar innervation, to the greater development of nerve fibres for the hind limb in the former animal.

Comparing the origin of the nerve fibres for the bladder with the origin of the nerve fibres for the descending colon and rectum—described in Part I.—we see that the difference is slight. So far as our experiments go, the bladder does not receive efferent fibres from the 13th thoracic or 1st lumbar nerves, and comparatively seldom from the 2nd lumbar. Thus the bladder appears to be supplied with efferent fibres by fewer lumbar nerves than the descending colon, rectum, and

internal anal sphincter. But as the effect produced by a given lumbar nerve is considerably greater on the end of the intestine than on the bladder, it is possible that the two viscera may be supplied by the same nerves, but that the upper lumbar nerves are only capable of causing a contraction of the bladder in exceptionally favourable circumstances.

A few lumbar fibres occasionally pass to the pelvic plexus and so to the bladder,—as has been described by Gianuzzi and Nussbaum—without traversing the inferior mesenteric ganglia and hypogastric nerves. The accessory hypogastric, when it occurs, contains few or no fibres for the bladder.

We give some additional—and we think conclusive—evidence for the view that both the lumbar nerves and the sacral nerves cause contraction of all the muscle fibres of the bladder, whether they are circular, oblique or longitudinal. Inhibitory fibres for the bladder are few, if indeed any exist.

The vesical arteries do not, as a rule at any rate, receive either constrictor or dilator fibres from the lumbar nerves; nor is there satisfactory evidence that they receive vaso-motor fibres from the sacral nerves. Micturition, normally, is only produced by two nerves out of the five to seven which have efferent vesical fibres. These are in nearly all cases the 2nd and 3rd sacral in the cat and dog, and the 3rd and 4th in the rabbit; the anterior of these two nerves only causes micturition on the cessation of the stimulus, since it causes also contraction of the striated muscles of the genito-anal apparatus.

The lumbar nerves have a bilateral action on the bladder owing to a decussation of fibres in the inferior mesenteric ganglia.

The sacral nerves have also a bilateral action, though the contraction on the side of the bladder opposite to the nerve stimulated is very slight compared with the contraction on the same side. The stimulus here probably spreads directly through the plexus of unstriated muscle.

PART III. THE EXTERNAL GENERATIVE ORGANS.

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Origin and Course of Inhibitory Fibres, p. 117.
p. 119. Summary.

THE parts of the generative organs with which we are concerned here are those developed from the uro-genital sinus and the skin surrounding the uro-genital opening. In the male these parts are the penis, the scrotum, and in some cases other parts of the skin in the genital region. In the female, they are the vulva and the genital skin; in the term vulva is included the vestibule stretching a short distance above the urethral opening. In human anatomy the vagina is commonly included in the external generative organs. It is well known, however, that developmentally it belongs to the internal generative organs, and we shall show later that it belongs to them also by its innervation.

PREVIOUS OBSERVATIONS[1].

Eckhard was the first to show that certain nerves when stimulated cause enlargement of, and increase of blood-flow through, the penis.

[1] See Foot-note on the next page.

These nerves he called the nervi erigentes. He found by dissection that in the dog they arose from the 1st and 2nd sacral nerves, and occasionally from the 3rd sacral nerve also. The experiments of Goltz showed the existence of an erector centre in the lumbo-sacral region of the spinal cord. Gaskell determined that the erector fibres left the spinal cord by the anterior and not the posterior roots, and in the rabbit by the anterior roots of the 2nd and 3rd sacral nerves. In the dog, Morat found them in the anterior roots of the 1st and 2nd sacral nerves. Langley described stimulation of the sacral nerves in the vertebral canal as producing sometimes dilation and flushing, sometimes contraction and pallor of the vagina (vulva). In the rabbit the former effects were most prominent with the 3rd and 4th sacral nerves, the latter with the 1st and 2nd sacral. The innervation of the penis was the same as that of the vulva; the sacral nerves causing either protrusion and flushing of the penis, or retraction and pallor. Sherrington observed in the monkey contraction of the external sphincter of the vagina on stimulating the 2nd and 3rd sacral nerves, the 3rd had usually a greater effect than the 2nd. The 1st sacral gave no certain result, but in three experiments out of sixteen it may have caused some contraction. In two experiments on the male, the 2nd and 3rd sacral nerves produced moderate, and the 1st sacral slight erection. In the cat, strong contraction of the external sphincter of the vagina was produced by the 1st and 2nd sacral, the 2nd giving usually a rather greater effect than the 1st. He states also that turgidity was produced by the 1st and 2nd sacral, and slight turgidity by the 7th lumbar nerve. Nikolski differed from Eckhard as to the result obtained by excitation of the anterior ramus of the nervus erigens in the dog, i.e. the ramus from the 1st sacral nerve. He found a vaso-constrictor, instead of a vaso-dilator effect. François-Franck states that this ramus is capable of producing either action.

[1] Eckhard. *Beiträge zur Anat. u. Physiol.* III. 123. 1863. (This Article gives an account of earlier observations); IV. 69. 1869; VII. 67, 115, 196. 1876.
Goltz. *Arch. f. d. ges. Physiol.* VIII. 460. 1874.
Nikolski. *Arch. f.* (*Anat. u.*) *Physiol.* 1879. 209.
Gaskell. *Journ. of Physiol.* VII. 1. 1886.
Gaskell. *Proc. Physiol. Soc.* Jan. 1887. (*Journ. of Physiol.* VIII.)
Morat. *Arch. de Physiol. norm. et path.* 1890.
Langley. *Proc. Physiol. Soc.* Dec. 1890. (*Journ. of Physiol.* XII. 1891.)
Sherrington. *Journ. of Physiol.* XIII. 675, 686. 1892.
Langley and Anderson. *Journ. of Physiol.* XVI. 417. 1894.
François-Franck. *Arch. de Physiol. norm. et path.* 1895, 122, 138.

Eckhard described erection as being produced in the rabbit, but not in the dog, by stimulation of the hypogastric nerves. Langley and Langley and Anderson found that these nerves sometimes contained constrictor fibres for the external generative organs. The fibres arose from the lumbar nerve-roots—about the 2nd to the 5th. François-Franck in the dog noticed chiefly a vaso-dilator effect on the vessels of the penis on stimulating the hypogastric nerves, but occasionally a vaso-constrictor effect was produced. Some of the branches running from the sympathetic to the inferior mesenteric ganglion had a similar effect.

Vaso-constrictor fibres for the penis were found by Eckhard in the nervus dorsalis penis. Gaskell suggested that the n. pudendus communis received its vaso-motor fibres from the grey rami of the sympathetic ganglia. Langley found constrictor fibres for the external generative organs of the male and female to arise from the lumbar spinal cord. The fibres—except a few going to the hypogastric nerves—ran down the sympathetic to the sacral ganglia, there became connected with nerve-cells and continued their course first in the grey rami of the ganglia and then in the sacral nerves.

The Upper Set of Nerves in the Male.

The general effects of stimulating the upper set of nerves are the same in the rabbit, the cat, and the dog. And in all cases unilateral stimulation has a bilateral visceral effect, though the effect is greater on the stimulated side. The effects are:

1. *Contraction of blood vessels.* The contraction of the arteries of the penis, both large and small, is very great. This is most readily seen in the dog; the large arteries which run on either side of the body of the penis contract to such an extent that the pulse in them disappears. The contraction of the arteries causes, of course, pallor, and decrease in size of the penis, varying according to the state of distension at the time.

There is also some contraction of the arteries of the whole genital skin. In the rabbit, the vessels in the median surface of the scrotum are clearly seen through the skin. On stimulating the upper set of nerves, the skin becomes slowly quite pale. In the rest of the genital skin of the rabbit, and in the whole region in the cat, the vessels are only imperfectly or not at all seen. But if the skin be cut, the blood-flow may be made slower by stimulating the upper set of nerves. The

contraction of the skin vessels is much less prompt than that of the vessels of the penis; and the upper lumbar nerves often have no visible effect upon the skin vessels, though causing distinct pallor of the penis.

2. *Contraction of the retractor muscle of the penis.* This muscle is well known in many animals; a sketch of it in the dog has been given by Eckhard (*op. cit.* 1863), who speaks of it as the 'Afterruthenband.' It is a thin band of longitudinally arranged unstriated muscle (15 to 20 centimetres long in a spaniel, weighing about 15 kilos), inserted at the attachment of the prepuce, and continued backwards in the middle line over the ventral surface of the corpus spongiosum and bulbus urethræ. In this part of its course the retractor runs in a loosely attached sheath, and is apparently a single strip of muscle, though easily divided into two halves near the bulb. Two small slips of the bulbo-cavernosus muscles in the dog accompany the retractor for the distance of 2 to 3 centimetres towards its insertion, and then join ventrally, forming a sling through which the retractor passes. Behind the bulb the muscle divides into two slips which pierce the external sphincter of the anus, passing one on either side of the gut; some of the fibres of either slip are continuous with a portion of the caudo-cavernosus muscle of the same side, others are attached to the urethra immediately behind the bulb, and a few pass dorsally to the urethra in the recto-urethral septum and may be traced as far as the prostate (see Fig. 1). The connection between the retractor and the caudo-cavernosus muscle is not easily seen in the dog but is obvious in the cat.

A few striated muscle fibres are found in the retractor in the cat and dog; they are derived chiefly from the external sphincter of the anus, but a few fibres probably pass into the retractor from the bulbo-cavernosus muscles.

The retractor muscle is well adapted for determining which lumbar nerves have an effect on the penis; it is exposed by a simple skin incision, and its contraction is easily seen. With a little care, the circulation of blood in it can be seen under the microscope; the vessels become nearly empty on stimulation of the lumbar sympathetic or of (e.g.) the 3rd lumbar spinal nerve. Further, it may be cut at its insertion and freed from the corpus spongiosum; and thus a long slip of muscle obtained connected only at its base. The slip contracts powerfully on stimulating the upper set of nerves. The fibres from these nerves run to the muscle, chiefly, at any rate, by way of the nervus dorsalis penis. In the *cat* the retractor muscle is more embedded in connective tissue than it is in the dog, and

until dissected out, it is seen in outline only. When it contracts it causes a marked dorsal curving of the penis. In the *rabbit*

FIG. 1.

Course and connections of the Retractor Penis of the Dog. Natural size.

there is no obvious muscle homologous with the retractor in the dog and cat.

It will be convenient to describe here some other unstriated muscles of the genito-anal region.

Frankenhaüser[1] indicates in Fig. 2 Pl. II. a muscle in the rabbit called by him the 'attractor' recti: this muscle arises from the 1st coccygeal vertebra between the two sacro-caudal muscles (m. flexores caudæ of Krause), and at its origin is composed of a single thin layer of unstriped muscle, which soon divides into two portions, homologous with the caudo-cavernosus and caudo-analis muscles described and drawn by Straus-Dürckheim[2] in the cat. In the cat and dog these muscles arise as in the rabbit between the sacro-caudal muscles as a single thin layer composed of unstriped fibres, but from the 2nd coccygeal vertebra. The anterior portion, forming the caudo-cavernosus muscle, is partly attached to the bulb and partly continued into the retractor penis; the lower portion, forming the caudo-analis muscle, passes under the external sphincter of the anus, and is attached to the anal skin. In the rabbit, the caudo-cavernosus muscle is very slight, and passes to the prepuce; the caudo-analis muscle, however, is well marked and distributed to the anal skin. The unstriped muscles of the genito-anal skin are also connected in the rabbit with a strong band of unstriped muscle, which extends from the urethral bulb to the seminal vesicles, running in the recto-urethral septum.

3. *Contraction of the intrinsic muscles of the penis.* In the cat and rabbit, when the body of the penis is cut open, it is seen that its whole musculature contracts on stimulation of the lumbar set of nerves. Retraction is thus produced when there is no blood-flow through the penis and in the cat when the retractor muscle has been cut away. The movement brought about by the contraction of the blood vessels, of the intrinsic muscles of the penis, and of the retractor muscle, working together, is considerable, and is conspicuous on external observation.

In the dog, the upper set of nerves cause slow movements in the penis itself, apart from that caused by the retractor muscle, but to these we have not paid much attention; the most obvious movement is the shrinking of bulbus urethræ. Externally very little change is seen in the dog after paralysis of the striated muscles: the thick skin hiding the effects which occur.

4. *Contraction of the unstriated muscles of the genital skin.* This corresponds with the contraction of the anal skin, which we have

[1] *Jenaische Zeitschr. für Medicin*, II. 1866.
[2] *Anatomie du Chat.* (Paris), 1845, pp. 319, 326.

mentioned in Part I. It is marked in the rabbit, much less in the cat, and slight only or absent in the dog. In the rabbit, the body of the penis lies underneath the end of the rectum, and both project from the limb muscles—an arrangement which does not exist in the cat or dog; the two or three centimetres of skin which cover the body of the penis move dorsally and anteriorly on stimulation of the lumbar set of nerves.

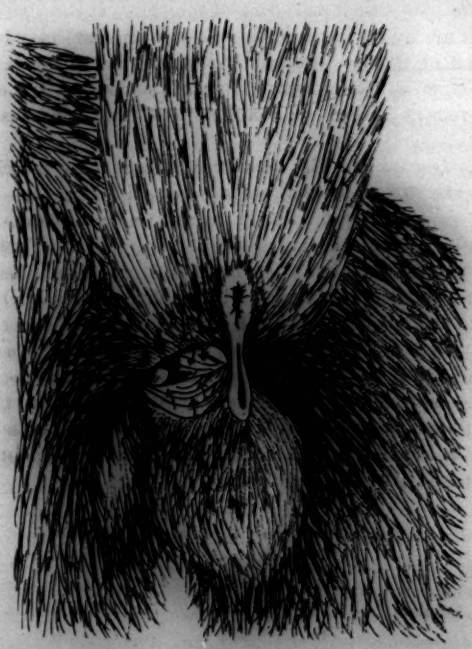

FIG. 2.

Diagram to show movement of genito-anal skin muscles in the rabbit produced by stimulating the upper set of nerves.

The skin over the end of the rectum moves at the same time ventrally and anteriorly, thus the body of the penis and the end of the rectum are brought nearer together along their whole length (cp. Fig. 2). With this there is contraction of the prepuce, which moves a little posteriorly, and closes over the retracting penis. The movement

of the prepuce may be distinct, when the penis, being already small, undergoes no further retraction. In the cat, there is contraction of the prepuce and a less obvious movement of the skin towards the anus.

5. *Contraction of the scrotum.* This comes under the previous section, but it is convenient to mention it separately. The movement in the rabbit is slight, and easily overlooked; it is obvious in the cat and dog, causing strong puckering of the skin. It must be remembered that some movement in the rabbit (less in the cat) may be caused by contraction of the vas deferens, and strong movement in the dog by contraction of the cremaster. Here of course we are only dealing with movement caused by contraction of the skin muscles (tunica dartos).

6. *Contraction of the cremaster.* Since this muscle does not belong to the visceral system, we have not paid very much attention to it. And we will give at once the results we have obtained as to the origin and course of the nerve fibres supplying it. First we should mention that Sherrington (*op. cit.* p. 683) found in Rhesus that the cremasteric muscle was innervated by the 2nd and 3rd lumbar nerves in eight cases and by the 3rd and 4th lumbar nerves in one case. In the cat and rabbit we have not seen any cremasteric contraction on stimulating either the lumbar spinal nerves or the genito-crural. And microscopic sections of the spermatic cord do not show the presence of any striated muscle. We conclude that in the cat and rabbit the cremaster is absent. In the dog we have made experiments with the results given in the following Table:

Origin of Nerves to Cremaster in Dog.

Exp.	$\frac{8}{11}$	10	4	7	5
I Lumbar	0	—	—	0	—
II „	0	—	—	0	—
III „	G	G	—	0	—
IV „	0	0	G	G	—
V „	—	0	0	0	0
Arrangement of Nerves	Markedly anterior	Med.[1]	Med.[1]	Post. (*a*)	Post. (*b*)[2]

[1] No. 10 was a little above and No. 4 was a little below the strictly median type.
[2] 6 Lumbar nerves only, the nerve below the 13th thoracic is in this Table counted as the 2nd lumbar, and the 13th thoracic as the 1st lumbar.

G indicates a good contraction.

It will be noticed that in Exps. 7, 8 and 11 at least, the cremaster received its motor fibres from a single nerve, either from the 3rd lumbar nerve or from the 4th. It is however possible that with a strictly median arrangement of nerves, the nerve fibres for the cremaster may be divided between the 3rd and 4th lumbar nerves.

Since the scrotal contraction is strong in the dog, it is possible, though not very excusable, to mistake it for the cremasteric contraction. Hence it is advisable to lay bare the spermatic cord near the epididymis, and hold it between finger and thumb; the contraction of the cremaster is then easily felt.

Unilateral stimulation gives unilateral contraction of the cremaster, the fibres run in the genital branch of the genito-crural nerve. We may mention that the genito-crural nerve contains non-medullated nerve fibres, but we have not seen any visceral effect on stimulating its peripheral end, either in the dog, cat or rabbit.

We have not found any satisfactory evidence of the presence in the lumbar nerves of vaso-dilator or inhibitory nerve fibres for the external generative organs, either in the male or female. We have found once or twice, as one of us found earlier, a slight flushing of the penis or vulva on stimulation of the upper lumbar nerves, and of the upper part of the lumbar sympathetic, but we are inclined after repeated experiments, to attribute the rare flushing to other causes than a stimulation of vaso-dilator fibres. On this point we shall later say something more.

After section of the lumbar nerves, or after section of the lumbar sympathetic in its lower region, there is usually more or less erection; obviously owing to a removal of tonic impulses to the arteries and unstriated muscle of the external generative organs.

Origin from the Spinal Cord. In the rabbit we have made a considerable number of experiments, stimulating the sympathetic trunk from above downwards (cp. Part I. p. 78). Sometimes there was a slight effect on stimulating just below the 2nd lumbar ganglion; a distinct though still weak effect was commonly obtained on stimulating below the 3rd lumbar ganglion; the effect was greater below the 4th, and greater again below the 5th lumbar ganglion. From this point down to the end of the sacral region, differences in the degree of action were difficult to determine. These experiments showed that constrictor nerves for the external generative organs commonly arise from the 2nd, 3rd and 4th (and possibly lower) lumbar nerves, and occasionally from

the 1st lumbar nerve also. Some similar experiments were made in the cat and with similar results.

We have also made more direct observations by stimulating the lower thoracic and the lumbar nerves in the vertebral canal. The results of these experiments we give in the Table on page 95. The symbols S. M. etc. are used with the same meaning as in earlier Tables (cp. Part I. p. 84). Unless otherwise mentioned in the Foot-notes to the Table, the effects observed in the cat and rabbit were, retraction and pallor of the penis, contraction of the skin muscles; and in the cat, contraction of the scrotum also. The body of the penis was not as a rule exposed[1] except in the dog, so that the changes were all observed on the structures visible externally. It may be noted that in these conditions, the upper effective spinal nerves caused slight pallor with little or no contraction of the penis. In the dog, contraction of the exposed retractor muscle, of the dorsal artery, and of the scrotum, were the chief points observed.

Upper Set of Nerves in the Female.

In their nature, the effects produced by the lumbar set of nerves in the female are, *mutatis mutandis*, the same as in the male. They are (1) pallor of the clitoris and of the mucous membrane of the vulva, with slight retraction of the clitoris. (2) Contraction of the vulva. This is much more obvious in the rabbit than in the cat and dog. In the rabbit the contraction is least just inside the vulvar orifice. The contraction of the vulvar orifice itself may be considerable. (3) Contraction of skin muscles, drawing the vulva dorsally. In the cat and dog there is a slight movement only of the vulvar orifice towards the rectum. In the rabbit the skin movement is fairly strong and is similar to that in the male (cp. Fig. 2, p. 91), the vulva being moved dorsally and somewhat anteriorly towards the rectum.

In most cases the effects are distinctly less than in the male, and sometimes they are very slight, especially in the cat and dog.

Our experiments on the effects of stimulating the lumbar nerves gave the results shown in the Table on page 96. In the cat pallor of the mucous membrane was chiefly observed, in the rabbit pallor of the mucous membrane and contraction of the genital skin.

[1] In the cat, it was exposed in Nos. 63, 64; in the rabbit in No. 37.

Origin of Nerves to External Generative Organs in the Male.

	Dog						Cat									Rabbit						
Exp.	11[1]	8[1]	10	4	7[2]	5	8	3	25[4]	1	32	7	4[5]	63[6]	64[7]	38	11	30	27	37	23	31/32	14	24	26
XII Thoracic	S	—	—	—	—	—	—	—	—	—	—	0	0	—	—	—	0	0	—	—	—	—	—	—	—
XIII ,,	M	M	—	—	M[2]	—	—	—	—	S[2]	—	?S[2]	?0	—	—	—	S[8]	?0	—	—	—	—	—	—	—
I Lumbar	S	S.M	—	—		—	—	—	—	S[2]	—	?S[2]	?0	0	—	—	S[8]	?0	0	0	—	—	—	—	—
II ,,	G	G	—	—	M	—	—	S	S	S>I	—	S	C	?0	?S	—	S[8]	?0	S	S	—	—	?S	—	—
III ,,	G	G	G	—	G	—	G	G	M	M	—	G	C	G	M.G	M	S.M	S.M	G	M.G	G	—	S	—	S
IV ,,	0	0	S.M[2]	G	S.M	—	S	—	M	M	G	M	C	G	G	G	G	G	G	G	G	—	G	G	G
V ,,	0	—	0	0	0	0	—	—	0	0	0	?S	0	M	G	S.M	M	G	—	G	G	G	G	G	G
VI ,,							—	—	—	0	0	0	0	—	—	—	0	0	—	0	0	—	0	0	—
Arrangement of Nerves		Ant.		Med.	Med.	Post. (a)	Post. (b)			Anterior		Ant. to Med.	—	Med.	P. (a)		Posterior (b)		A.	A. to M.	Med. to Post.		Post.		

[1] The XIIth and XIIIth Th. caused contraction of the retractor muscle, some contraction of the preputial artery, little or none of the dorsal artery of the penis, nor of the scrotum.
[2] No effect seen on the scrotum.
[3] Six lumbar nerves only present, the 13th thoracic was counted as the 1st lumbar.
[4] Scrotum not observed. Rami to inferior mesenteric ganglia cut.
[5] The arrangement of the nerves was not sufficiently noted; it was either Ant. to Med. or high Median.
[6] The effect on the penis is here given, the origin of the nerves for the scrotum was different, viz. II L. = M; III L. = G; IV L. = G; V L. = 0.
[7] The effect on the penis is here given, the origin of the nerves to the scrotum was, II L. = ?S; III L. = G; IV L. = G; V L. = Trace.
[8] Pallor only obtained.

S.M indicates that the effect varied from Slight to Moderate; M.G that it varied from Moderate to Good.

Origin of Nerves to External Generative Organs in the Female.

	Cat									Rabbit				
Exp.	2	33	37	27	35	10	14	26	9		35	9	19/20	25
XIII Thoracic	0	0	0	—	?0	?0	—	—	—	XII	0	—	—	—
I Lumbar	?S	?0	?0	0	?0	?S	0	0	?0		0	0	—	—
II ,,	S	S	S	?0	—	M	S	0	S		0	0	—	—
III ,,	S	M	M	C	—	M	S	S	M		M	S	M	—
IV ,,	S	S.M	M	C	—	M.G	M	G	G		G	G	M	G
V ,,	0	0	0	?0	—	0	S	G	—		S	M	—	—
VI ,,	0	0	0	—	—	—	—	0	0		0	—	—	—
Arrangement of nerves	Ant.	Ant. to Med.		Median				P.(a)	P.(b)		A. to M.	Median		P.

Course taken by the Upper Set of Nerve Fibres.

The course taken by the lumbar nerve fibres to the external generative organs is the same in the male and female, but since the effects are generally much more obvious in the male than in the female, we have taken the former in nearly all cases when comparatively slight effects were to be observed. The results given in this section refer to both rabbit and cat unless otherwise mentioned.

The nerve fibres pass from the spinal nerves by the white rami and run to the lumbar sympathetic chain. Stimulation of the sympathetic just above the 1st sacral ganglion produces maximal effects. An experiment bearing on this point has been given in Part I. p. 79. When both sympathetics are cut just above the 1st sacral ganglia, stimulation of the lumbar nerves has a comparatively slight effect; often, indeed, unless special precautions are taken, no effect is to be seen. Hence the great majority of the upper set of fibres run to the external generative organs by way of the sacral sympathetic chain.

Course by pudic nerves. It is equally clear that the great majority of the fibres leave the sympathetic chain by the grey rami of the sacral ganglia, run to the sacral nerves, and reach the periphery by way of the dorsal nerves of the penis or clitoris, the genito-anal and the scrotal nerves. This can be shown in several ways.

(*a*) The sympathetic may be cut above and below each sacral ganglion and the piece of nerve above the ganglion, the ganglion, or the grey ramus itself, stimulated. Proceeding in this way, we have obtained retraction of the penis, and contraction of the tunica dartos from the 1st, 2nd and 3rd sacral ganglia in the cat and dog, and from the 2nd, 3rd and 4th

sacral and 1st coccygeal ganglia in the rabbit. This method is troublesome and we have not attempted to determine by it, the variations in distribution which correspond with the anterior, median, and posterior arrangements of the spinal nerves.

(*b*) The grey rami of the sacral and coccygeal ganglia may be cut, and the sympathetics stimulated just above the 1st sacral ganglia. Such stimulation gives either no effect or a very slight one. This method is still more troublesome than the first, and a considerable reduction of the normal blood flow through the external generative organs is unavoidable.

(*c*) The pudic nerves may be cut and the sympathetics stimulated as in the previous case. Little or no effect is then produced. When all the branches of the pudic nerve are cut on one side, say the right, and the sympathetic on the same side is stimulated, there is still some action on the external generative organs, though more or less confined to the left side. The bilateral action is due to the connection which exists in the sacral region between the sympathetic trunk on the two sides. The lumbar fibres, which descend the sympathetic on one side, run in part to the sacral ganglia of the opposite side.

By a trifling modification of method (*c*), the distribution of the sympathetic fibres contained in each branch of the pudic nerve can be determined. Instead of cutting all the branches at once, they are cut in succession; the sympathetic being stimulated after each branch is cut. We have not worked this out in detail, but in the experiments made the results were as follows:

In the rabbit, section of the nervus dorsalis penis or clitoridis on both sides abolished the effects of the sympathetic on the penis (or clitoris and vulvar mucous membrane) and greatly diminished the effect on the prepuce; section of the superficial genito-anal nerve had no appreciable effect; section of the deep genito-anal decreased greatly the effect on the anal and genital skin.

In the cat after section of the combined nervus dorsalis penis and genito-anal nerve, stimulation of the sympathetic had little or no effect on the penis or skin; but still had a strong effect on the scrotum; after section of the perineal, or simply of the scrotal branches, the sympathetic had no effect on the scrotum.

Course by hypogastric nerves. A certain number of the upper set of fibres—comparatively very few—run to the generative organs by way of the hypogastric nerves. We have said that sometimes slight retraction and pallor of the penis are obtained by stimulation of the lumbar spinal

nerves after both sympathetic trunks have been cut just above the 7th lumbar ganglion. There may be a slight effect on the prepuce, but we have not seen any effect in the skin elsewhere, including that of the scrotum.

Stimulation of the peripheral end of one or of both hypogastric nerves, generally causes some retraction and pallor of the penis, and, if the penis be cut across, some decrease in blood flow. The effect is very variable in extent. In not a few cases, we have found no obvious effect; but in these cases, the body of the penis was not exposed and its blood vessels were already moderately contracted. In all doubtful cases it is advisable to expose the body of the penis, and to stimulate the genital branch of the nervus erigens before stimulating the hypogastrics. Very marked effects may then be observed. The hypogastrics give similar but less obvious effects upon the vulva. Removal of the sacral spinal cord does not prevent their action.

Aberrant Course. Besides the two courses described above by which the lumbar spinal nerves send fibres to the external generative organs, there are various other possible courses.

In the first place, the aortic plexus sometimes gives off a filament to the pelvic plexus, and this may contain a few fibres for the generative organs. Thus if both hypogastric nerves are cut about an inch below the inferior mesenteric ganglia, and both sympathetic nerves cut just above the 7th lumbar ganglia, and then both sympathetics are stimulated from the 3rd to the 6th lumbar ganglia, a slight retraction of the penis is occasionally obtained; the retraction is no longer obtained after tearing away the aortic plexus. The fibres taking this course are undoubtedly homologous with the hypogastric fibres.

Secondly, a few fibres may leave the sympathetic at or below the 7th lumbar ganglia, and run in some other course than the pudic nerves. In order to determine this, we have had to make a considerable number of experiments.

At first, we exposed the branches of the pudic nerve from the ventral surface. Both sympathetics were stimulated just above the 7th lumbar ganglia, and the usual contraction noted. It was somewhat less than normal owing to the exposure of the parts during the dissection of pudic nerves. The pudic nerves were then cut on both sides, and the sympathetic again stimulated. In six such experiments on rabbits (one being female), we were unable to observe any effect from stimulation of the sympathetic after section of the pudic nerves.

We then exposed the pudic nerve from the dorsal surface. Since it

FIG. 3. Structures seen when the pudic nerve of the Rabbit is freely exposed from the dorsal surface. Twice the natural size. The nervus cutaneus femoris posterior, superficial genito-anal, and scrotal nerves, are represented for the sake of clearness as forming one trunk, in fact they are only loosely attached to one another. Posterior arrangement of nerves.

requires considerable experience to recognize readily the various origins and branches of the pudic nerve, when dissected without unnecessary exposure, we give below sketches of the surroundings of the pudic nerve in the rabbit and cat, when it is exposed in the greater part of its course from the dorsal surface.

In the rabbit, after section of the pudic nerves, the sympathetics at the level of the 7th lumbar ganglion gave no effect in three females and one male; but in one male, the sympathetics caused slight retraction and pallor of the penis, without any retraction of the genito-anal skin. In these experiments, the tissues were observed externally.

FIG. 4.

Structures seen when the pudic nerve of the cat is freely exposed from the dorsal surface. Natural size. Posterior arrangement of nerves.

Five experiments were made on cats, all being males. In three of these, the sympathetics had no effect after section of the pudic nerves. In the other two, the sympathetics caused slight retraction of the penis, provided the penis was in a state of considerable erection at the moment of the sympathetic stimulation.

Although we have only found the lower lumbar sympathetic to be effective, after the pudic nerves have been cut, in three out of fourteen cases; yet we think that usually a few nerve fibres for the penis (or clitoris) do run by way of the lower lumbar sympathetic chain without passing to the sacral nerves. The frequent failure to show them experimentally was we think due to insufficient attention to the conditions of success. We append a brief account of one of the successful experiments (cp. also Part II. p. 76).

Protocol III[1]. *Cat. Effect of stimulating the sympathetic below the 7th lumbar ganglion after section of the pudic nerves.* Chloroform, then A. C. E. by tracheal tube. Pudic nerves exposed dorsally on both sides. Cut through lower part of linea alba, double tie and cut femoral vessels. Tie and cut sympathetic trunk on each side, just below the 7th lumbar ganglion; isolate each up to the 1st sacral ganglion.

Stim. lower cut end of left sympathetic. Strong contraction of tunica dartos (both sides), strong retraction and good pallor of penis, strong erection of hairs of tail.

Stim. right sympathetic. Effects like those of left sympathetic.

Cut on both sides the exposed pudic nerves, not including the perineal branches.

Stim. left sympathetic. Strong contraction of tunica dartos, and erection of hairs, no effect on penis.

Stim. right sympathetic. Effect as on left side.

Cut on both sides the perineal nerve.

Stim. both sympathetics. No effect seen on penis, prepuce, scrotum or bladder. Strong erection hairs of tail.

Tie and cut both hypogastric nerves just below the inf. mesenteric ganglia.

Penis now projects, are some spontaneous variations in size; when a considerable excess of the A. C. E. mixture is given there is some shrinking of the penis.

Stim. left hypogastric. Good contraction of bladder, vas deferens, seminal vesicle, slow contraction of internal anal sphincter. No certain effect on the penis.

[1] Protocol I. in Part I. p. 78, and Prot. II. in Part II. p. 76.

Tie and cut medio-posterior branch of nervus erigens on the left side. Stimulate this branch of the nervus erigens to obtain projection of penis.

Stim. hypogastrics. Distinct retraction of penis.

Cover up for ten minutes. Penis again projected.

Stim. hypogastrics. Retraction of penis.

Stim. sympathetics. No certain effect on penis, strong erection of hairs of tail.

Stim. nervus erigens to obtain projection, then stim. sympathetics. Slight retraction.

Cover up for ten minutes. Penis projected.

Stim. sympathetics. Slight retraction.

Stim. hypogastrics. Slight further retraction.

The exact course taken by these fibres is not easy to determine. We do not think they run by the sacral grey rami to the spinal nerves and then peripherally in the nervus erigens, since such fibres should not be paralysed by nicotin (cp. Part V.) and after nicotin has been given, stimulation of the nervus erigens near its origin has no effect on the generative organs. Nikolski described in the dog a filament running from the 7th lumbar ganglion to that ramus of the nervus erigens which arises from the 1st sacral nerve. We have sometimes found such a filament running direct to the pelvic plexus near the entrance into it of this anterior ramus[1]. Some of the upper lumbar fibres run we think in this filament, or in a corresponding filament from the 1st sacral ganglion. These, like the fibres which branch off from the aortic plexus, we take to be homologous with hypogastric fibres. But as each sympathetic ganglion gives off some fibres to the neighbouring arteries, it is possible that a few lumbar fibres may run to the generative organs by way of the arteries supplying them, without traversing the pudic nerves, or the pelvic plexus.

But whatever the course may be, it must be borne in mind that the total number of fibres which take any other course than that of the pudic nerves or the hypogastric nerves is extremely small.

Question of inhibitory fibres in the hypogastric nerves.

We have already said that we have found no satisfactory evidence of the presence in the lumbar spinal nerves of vaso-dilator fibres for the generative organs. Repeated experiments on stimulation of the

[1] In one case (dog) a filament was given off by the 7th lumbar ganglion to the lower part of the hypogastric *nerve*.

nerves in the peripheral part of their course, namely, in the sympathetic trunk at the junction of the lumbar and sacral region, and in the hypogastric nerves, have not given us any indication of the existence of erector fibres in them. The absence of erector action on stimulating the hypogastric nerves was the more surprising, since Budge has described erector action from the hypogastrics in the rabbit, and François-Franck from the hypogastrics in the dog. Budge observed the action directly. It is not impossible that the erection observed by him should have been brought about reflexly (cp. Part I. p. 69), or by an escape of current to the sacral erector fibres in the pelvic plexus. François-Franck deduced the presence of erector fibres, from the curves obtained by the graphic method. The interpretation of the curves does not seem to us to be by any means a simple matter. It is hardly necessary to criticise them at present, for if the hypogastric nerves have any such dilator effect as is attributed to them by François-Franck, it must be visible to the eye, when the penis is exposed. In the rabbit and cat taken together, we have in more than thirty cases, and in the dog in seven cases, observed the external generative organs during stimulation of the hypogastric nerves. In the cat and rabbit, there was, as a rule, some degree of contraction. But we have not once seen dilation of the blood vessels or erection. On the lumbar sympathetic, our experiments have been more numerous and the results similar. Since slow rhythmic shocks are held by some to be more effective on vaso-dilator than on vaso-constrictor nerves, we have occasionally stimulated the lumbar spinal nerves and the hypogastrics with induction shocks repeated once a second, instead of with tetanising currents; constriction and not dilation was still obtained. When stimulating the sacral nerves—or the nervi erigentes—we have no difficulty in obtaining erector action. We think, then, that the evidence does not warrant the view that there are erector nerve fibres in the lumbar spinal nerves, or in the hypogastrics.

The lumbar nerves or the hypogastrics, though unable normally to cause micturition, often force a little urine into the upper part of the urethra. In such case the urethra may contract reflexly. And the reflex may affect other genital muscles, and cause some movements of the penis. Occasionally we have seen fairly strong contraction of the whole genito-anal striated muscles to be set up in this way. It is possible that similar contraction should be set up when the seminal vesicles force their contents into the urethra. Some movement of the penis may also be caused by the contraction of the muscles of the rectum.

The Lower set of Nerves.

Function in the Male. Some of the effects of the lower set of nerves are well known, namely:

(1) *Flushing and swelling of the penis.* With unilateral stimulation, the effect can usually be seen to begin on the stimulated side; thus in the rabbit, after curari, if the prepuce be held back, stimulation of the nerves on one side may cause the penis to project and curve towards the opposite side. A slight swelling of the organ easily escapes notice if its projection from the prepuce is alone looked for. In all doubtful cases, the prepuce should be cut through and the penis separated from the surrounding tissue as far as the limb muscles.

(2) *Contraction of the striated erector muscles,* viz. the recto-cavernosus, the ischio-cavernosus, the bulbo-cavernosus, Houston's muscle and the striated muscle of the urethra. In the rabbit there is also the pubo-cavernosus. In the cat, the bulbo-cavernosus on either side is prolonged along the ventro-lateral part of the penis, running on either side of the urethra, as far as the attachment of the prepuce. In the cat also, there is an elevator of the scrotum, which causes the scrotum to be drawn sharply towards the anus; this movement appears to aid considerably in the protrusion of the penis. Unilateral nerve stimulation causes the penis to be pulled forcibly towards the stimulated side. In the cat and rabbit, when the penis is freed up to the muscles of the limb, the upper set of nerves cause the penis to straighten with a jerk, and to shoot out a little (contraction of the bulbo-cavernosus). As a rule there is simultaneously a strong ejaculator urinæ action, a drop or two of urine being violently ejected. There is a similar spirt of blood, on stimulating the upper set of nerves after the penis has been cut through at the level of the attachment of the prepuce; this does not occur after curari has been given; it is clear that normally the contraction of the striated muscles must drive violently forward a few drops of blood into the anterior end of the penis.

Other effects are:

(3) *Inhibition of the retractor muscle.* This is most readily seen in the dog, and in the dog it is a very striking phenomenon. When the muscle is cut through at its insertion in the prepuce, and the connective tissue on either side is also cut through for 2 to 3 centimetres, stimulation of one of the effective lumbar nerves causes it to

contract and to be drawn more or less completely into the connective tissue sheath which covers it. When the cut end is lightly pulled with forceps, there is clearly felt resistance to extension, and it is one to two minutes before the (approximate) original length is obtained. When however the sacral nerves are stimulated immediately after the lumbar nerves, there is a period of 10 to 20 seconds, during which the resistance to extension is felt as in the first case, but then the muscle begins to yield, and it is, with very gentle tension, rapidly drawn out to full length. If the muscle is left untouched, it sometimes becomes obviously flaccid on stimulating the sacral nerves. It is not difficult in this manner to satisfy oneself of the presence of inhibitory sacral fibres, notwithstanding the movements caused by the simultaneous contraction of the genito-anal striated muscles. The effect of these can of course be abolished by curari. But in an experiment like that given above, some movement of the cut end of the retractor will be caused by certain of the unstriated muscles of the genito-anal apparatus apart from a direct action on the retractor. Thus, contraction of the recto-coccygeal muscle may press posteriorly the external anal sphincter and so allow the cut end of the retractor to move anteriorly (cp. Fig. 1, p. 89). The considerable swelling of the bulb of the penis has a tendency to force the cut end of the retractor backwards. The method by which the inhibition of the retractor muscle can be most conclusively shown is perhaps the following. In an anæsthetised dog, one of the effective lumbar nerves (say the 3rd) and the cauda equina are prepared for stimulation. Morphia and curari are then injected into a vein. The retractor muscle is cut at its insertion and tied by a thread, which is carried over a wheel to a weighted lever; the muscle is isolated as far as the posterior part of the bulb, here it is clamped with just sufficient firmness to prevent it sliding in the clamp, which can be done without stopping the passage of nervous impulses. The part of the muscle between the clamp and the tied end cannot be moved by the contraction of the rest of the muscle, or by any other muscle; it is practically isolated from the body except as regards its nerve-supply. Yet the relaxation on stimulating the cauda equina, is in favourable circumstances almost as obvious as the immediately preceding contraction on stimulating the lumbar nerve. On simultaneous stimulation of the lumbar and sacral nerves, so far as our scanty observations on this point go, the contractor effect overcomes the inhibitory, except when the stimulation of the lumbar nerve is very weak.

4. *Inhibition of the unstriated muscles of the genital skin.* Stimu-

lation of the lower set of nerves causes a movement of the skin—provided of course the fibres have some tone—in the opposite direction to that produced by the upper set of nerves (cp. Fig. 2). In the rabbit only, is the effect an obvious one; the skin which overlies the body of the penis moves posteriorly and ventrally, so that it and the lower end of the rectum become farther apart. The movement is to a considerable extent passive, due partly to the increase in the volume of the penis, and partly to the longitudinal contraction of the rectum, but it is partly due to inhibition of the skin muscles, since it may be seen when precautions are taken that other agencies shall have no effect. When curari is not given, the striated muscles at the base of the penis may counteract the inhibitory downward movement. In the cat, the lower set of nerves cause the prepuce and anus to move farther apart, but the movement is slight and whether it is due to inhibition of skin muscles we have not determined.

The sacral nerves have not, apparently, any inhibitory fibres for the unstriated muscle of the scrotum. In the rabbit the contraction caused by the lumbar nerves is so slight, that we could hardly expect a marked inhibitory effect from the sacral nerves. But in the cat, and still more in the dog, strong contraction is produced by the lumbar set of nerves, and nevertheless we have never been certain of any increase in the rate of relaxation on stimulating the separate sacral nerves or the cauda equina. Now and then we have thought there was a slight inhibition, but since in no experiment was the effect constant, or even frequent, we think it was simply the expression of normal variations in the relaxation rate.

Nor do we find that the sacral nerves contain vaso-dilator fibres for the scrotum; occasionally on stimulating these nerves in the rabbit there was slight flushing, but owing to its infrequency and inconstancy we think it was due to 'spontaneous' variations, and not to the stimulation of inhibitory sacral fibres.

All of the effects spoken of above are produced by nerve fibres which run from the spinal cord in the sacral nerve roots. Besides these, there may be seen, on stimulating the sacral nerves in the vertebral canal, other effects which, as we shall show later (p. 108), are not caused by nerve fibres issuing from the sacral spinal cord. The effects are, pallor and retraction of the penis, contraction of the prepuce and genital skin, contraction and pallor of the scrotum, in short, all the effects produced by the upper set of nerves. Thus on stimulating an

effective spinal nerve near its exit from the vertebral canal, there is commonly a struggle between two antagonistic actions; at times one, at times the other predominates, and a slight shifting of the electrodes may vary the degree or the nature of the predominance, hence the actual changes observed vary very widely. The contractor effects are more easily produced in the rabbit than in the cat, and more easily in the cat than in the dog.

Lastly we may notice an observation by V. Anrep and Cybulski[1]. They found in the dog that the nervus erigens caused normal swelling of the penis after intra-venous injection of 30 mgr. of atropin. We have injected up to 60 mgr. of atropin into the jugular of the rabbit, and find similarly that none of the actions of the sacral nerves on the external generative organs in male or female is appreciably affected.

Function in the Female. Here, as in the male, two groups of effects, antagonistic to one another, may be produced by stimulating the lower set of nerves in the vertebral canal, and as in the male, only the nerve fibres which have an inhibitory action run from the spinal cord in the sacral nerve roots. The inhibitory effects are: (1) Flushing of the mucous membrane of the vulva, and still more of the clitoris. (2) Dilation of the vulva. In the rabbit this is often very marked, it is greatest at the vulvar orifice and least immediately above the orifice; it is best seen when the vulva is held open in a state of slight tension. In the cat and dog, this effect is slight or absent. (3) Relaxation of the skin muscles around the vulva. In the rabbit, the whole surrounding skin moves ventrally, that over the end of the rectum moves dorsally; the movement corresponds with that we have described in the male.

The visceral motor effects are the same as those produced by the upper set of nerves (p. 94) and we need not repeat them.

Besides these effects there is of course contraction of what is usually called the external sphincter of the vagina and of the striated muscles which correspond with the striated genital muscles of the male. In the rabbit the external vaginal sphincter is situated towards the anterior portion of the vulva, and not near its opening. In the dog, on stimulation of the sacral nerves, the vulva is pulled dorsally so that the upper part disappears under a fold of skin.

[1] Hofmann and Schwalbe's *Jahresb.* 1884, p. 49.

Motor visceral effects of stimulating the sacral nerves in the spinal canal. The first point that demands attention is the motor visceral action (contraction of unstriated muscle fibres) given by the sacral nerves. It has been shown[1] that in certain cases the sympathetic fibres which run in a grey ramus to a spinal nerve can be stimulated by stimulating the spinal nerve in the vertebral canal. And we have shown above (p. 96) that the sacral sympathetic sends motor visceral fibres to the sacral nerves by way of the grey rami. There is then a certain likelihood that the contraction and pallor of the generative organs, which can be produced by stimulating the sacral nerves in the vertebral canal, are due to excitation of sympathetic nerve fibres. And this we find to be the case.

The obvious way of testing the matter is to stimulate the nerve roots as far as possible from the grey ramus, *i.e.* as near as possible to the spinal cord. When this is done, and proper precautions are taken to prevent the electric currents from spreading down the nerve, inhibition, *i.e.* erector action only is obtained. Some care, however, is required to obtain the nerve roots in the normal state of irritability so that weak shocks only need be used.

It is not absolutely necessary to take the nerve roots, for as a rule, weak stimulation of the nerves just peripherally of the posterior root ganglia gives inhibitory effects only; contractor effects begin to be seen as the electrodes are shifted still more peripherally; they are most marked when the nerves are stimulated at their point of exit from the bone, and stimulated with a strong current. In the cat, the erection of hairs in the local area of the nerve stimulated affords a useful indication that fibres of the sympathetic grey ramus of the nerve are being stimulated.

The question may be approached in another way. If the roots of the spinal nerves contain motor visceral fibres, they must run either in the pelvic visceral nerves or in the pudic nerves. But when the sacral nerves are stimulated, after section of the pudic nerves, inhibition and erection only are obtained; so that no motor fibres—or more strictly, no appreciable number of them—are contained in the pelvic visceral nerves (nervi erigentes) at their origin.

With regard to the other possible course of motor fibres, namely, the pudic nerves, we can compare the motor effect of any one branch with that of the sympathetic fibres which go to the branch. Thus suppose

[1] Langley. This *Journal*, xv. 203. 1893; xvii. 311. 1894.

in the cat all the branches of the pudic nerves are cut except the perineal branch on one side. If then the perineal nerve receives motor visceral fibres from the sacral nerve roots as well as from the sympathetic, it should be capable of producing with a weak stimulus a stronger contraction of the scrotum than that produced by a similar stimulus applied to the sympathetic trunk in the upper sacral region[1]. We do not find this to be the case.

Lastly we may note the action of nicotin on the sacral nerves. If the nerve roots contain motor visceral fibres for the external generative organs, they will—in accordance with the general law regarding visceral fibres—have nerve cells on their course, and the connections with the nerve cells will be paralysed by nicotin. In fact, when nicotin has been given, stimulation of sacral nerves in the vertebral canal still gives contraction and pallor in the generative organs and area, although it gives no trace of inhibitory or erector action.

We conclude then that the motor effects given by the sacral nerves are due to the stimulation of sympathetic fibres, and that the inhibitory effects only, are to be attributed to fibres issuing from the cord by the anterior nerve roots.

So far we have spoken broadly of the occurrence of motor visceral effects on stimulation of the sacral nerves. These effects are not however obtained with equal ease, nor with equal distinctness from each of the sacral nerves, nor are they necessarily obtained from all.

The ease with which the effects are obtained depends chiefly on the extent to which the fibres of the grey ramus can be stimulated by stimulating the spinal nerve in the vertebral canal. The longer the piece of nerve, which lies in the vertebral canal, the more easily and completely the grey ramus can be stimulated by stimulating the nerve. Thus the lower sacral nerves will give visceral motor effects, when stimulated a considerable distance from their point of exit, and with weak currents, whilst the 1st sacral in similar conditions gives little or no effect. The reason of this is that the grey rami of the lower sacral nerves penetrate the spinal canal in the nerve sheath before turning peripherally. The following diagrams will perhaps make the matter clearer.

A spinal nerve is shown passing out of an intervertebral foramen; *b* is the edge of the bones of the vertebræ; *p. pr. d.* is the posterior

[1] The argument of course would fail, if the sacral sympathetic sent any considerable number of fibres to the external generative organs by any other course than the pudics. (On this point cp. p. 98.)

primary division (dorsal division) of the nerve; *a. pr. d.* is its anterior primary division (ventral division); *symp. gang.* is the sympathetic ganglion; *gr. r.* is its grey ramus.

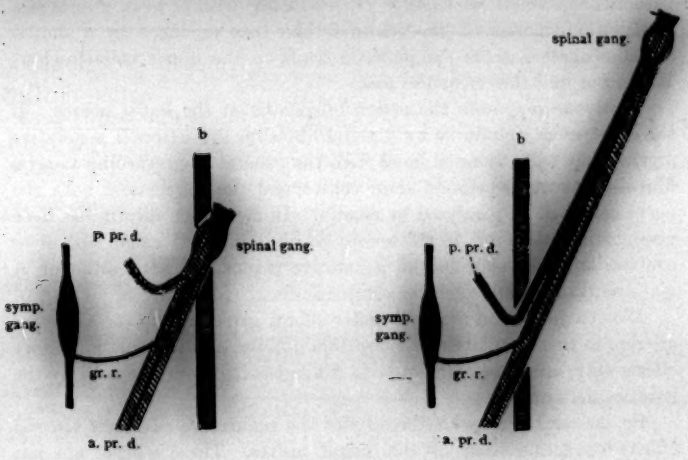

FIG. 5[1].

Fig. 5 A. is a diagram of the arrangement in the cervical, thoracic and lumbar regions. Here the grey ramus is outside the spinal canal and it will not be affected by moderate stimulation of the nerve roots inside the canal. When however the nerve is pulled into the spinal canal, and it is stimulated peripherally of its ganglion, there will be an escape current to the fibres of the grey ramus which run to the dorsal division of the nerve (*p. pr. d.*), and possibly also to those which run to the ventral division[2].

Fig. 5 B. is a diagram of the arrangement in the 2nd sacral nerve and of the nerves below it. Here the grey ramus can be stimulated in the spinal canal, and as the electrodes are passed down the nerve, the fibres to the dorsal division will first be stimulated, and then the fibres to the ventral division.

[1] Since the Figure is simply a diagram to show a special point, the fibres which run from the grey ramus to the dura mater are not inserted.

[2] Cf. Langley. This *Journal*, XV. 203. 1893; XVII. 306. 1894.

In the cat retraction and pallor of the penis and contraction of the scrotum can always be obtained by stimulating the 2nd sacral nerve near its exit from the sacral canal (cp. Exp. p. 114). The 3rd sacral nerve gives a moderate effect, when the arrangement of the nerves is posterior. The 1st sacral nerve gives little or no viscero-motor effect, unless a strong current is used, and then it is but slight, unless there is escape of current to the 2nd sacral nerve. If, however, the 1st sacral nerve is isolated nearly up to the sacral plexus, and the part which lies just outside the sacral foramen be stimulated, contraction of the penis and scrotum is readily obtained. We give an account of an experiment to illustrate this and some other points.

Protocol IV. *Cat* (*No.* 62). *Effects of stimulating the sacral nerves inside and outside the vertebral canal.* Nerves, median arrangement. Chloroform, later A.C.E. mixture. Expose sacral plexus on left side (cutting through ilium and edge of sacrum) so that the nerves are seen at their exit. Expose lower part of spinal cord, cut it through in mid-region of VIIth lumbar vertebra. Tie and cut on each side VIIth lumbar, I, II, III sacral and I coccygeal nerves a little above the posterior root ganglia, isolate them to their point of exit. Cut away bone at exit of I and II sacral, on the left side, and isolate them for half to three-quarters of a centimetre outside the sacrum. Stimulate the nerves with a tetanising current, secondary coil at 9 cm. of scale; shocks are distinctly felt on tip of tongue. The nerves on the right and left side were first stimulated near the posterior root ganglia. The results were:

VII Lumbar. Strong contraction of leg, toes widely separated, claws out. No effect on external sphincter, anus, or penis.

I Sacral. Strong contraction of leg, foot extended, toes bent a little backwards. Strong contraction of external anal sphincter and of elevator of scrotum. Moderate protrusion of penis, some flushing.

II Sacral. Toes bent. Moderate contraction of external anal sphincter, slight of elevator of scrotum. Penis a little protruded, some flushing. Contraction of ejaculator urinæ. Micturition after end of stimulation.

III Sacral. Strong micturition.

I Coccygeal. Tail movement only.

Cut through skin over penis in mid-ventral line to observe body of penis. Swelling of the penis is obtained from the Ist and IInd sacral nerves. The IIIrd sacral nerves (the two stimulated together) cause apparently a slight swelling, but the result is too inconstant to be quite certain about.

Separate body of penis from surrounding connective tissue and stimulate on left side Ist and IInd sacral nerves a little outside the sacral foramina.

I Sacral. At once, some protrusion and straightening of penis from contraction of its striated muscles (including ejaculator urinæ), then great retraction, shrinking, and flaccidity.

II Sacral. Rather less protrusion and straightening of penis, otherwise effects like those of the Ist sacral.

Cut on left side the filament sent by IIIrd sacral nerve to the nervus erigens. Stimulate the IIIrd sacral near its exit. There is apparently some retraction of the penis, but it is not sufficiently constant to be certain about.

Cut through the linea alba and observe the bladder, stimulating the sacral nerves on both sides.

I Sacral. No effect from either nerve.

II Sacral. Good contraction from both nerves.

III Sacral. Good contraction from right nerve, none from left (cp. above, strand to nervus erigens cut).

I Coccygeal. No effect from either nerve.

In the rabbit, the 4th sacral nerve, stimulated in the spinal canal near its exit, always gives strong viscero-motor effects; with an anterior arrangement of nerves, the 3rd sacral gives a moderate, and the 2nd sacral (usually) a slight effect; with a posterior arrangement of nerves, the 3rd sacral and the 1st coccygeal nerves give a moderate effect.

From the results of experiments on stimulation of the spinal nerves inside and outside the vertebral canal, and on stimulating the sacral ganglia or grey rami, we come to the following scheme as to the grey rami which supply the external generative organs, and as to their relative degree of action. We must state, that we have not made a sufficient number of experiments to be quite certain of all the points of this scheme; the doubtful points we have queried.

	Cat			Rabbit	
	Anterior	Median	Posterior	Anterior	Posterior
VII L.	0	0	0	0	0
I S.	G	G	↑M	↑S	0
II S.	G	G	G	M	↑S
III S.	↑0	0 or S	S M	G	G
IV	0	0	0	M	G
V	0	0	0	0	S M

It will be convenient to give here the several effects which we have observed on stimulation of the various branches of the pudic nerve in the male. In the female, corresponding results are obtained.

Rabbit. The *superficial genito-anal* causes strong contraction of the external sphincter of the anus and vagina, of the recto-cavernosus, the ischio-cavernosus and bulbo-cavernosus, and some contraction of the urethra. The only visceral effect it produces is a contraction in the posterior part of the skin over the rectum, and this is due to a filament it receives from the deep genito-anal. When the branches of the nerve are taken, good contraction of the external sphincter can be obtained from several of them.

The *nervus dorsalis* causes strong contraction of the pubo-cavernosus, of Houston's muscle and of the urethra. Its visceral effects are strong contraction of the intrinsic muscles of the penis, of the blood vessels of the penis; strong contraction of the prepuce, moderate contraction of the rest of the genital, but not of the anal skin.

The *deep genito-anal* causes some contraction of the bulbo-cavernosus and of the urethra near the bulb. Its visceral effects are contraction of skin muscles both of the anal and genital skin (cp. Fig. 2).

The *perineal nerve* causes slight contraction of the tunica dartos and of the blood vessels of the scrotum.

Cat. The *nervus dorsalis* has a similar action of that in the rabbit except that it has less effect upon the body of the penis.

The *genito-anal nerve* has the combined action of the superficial and deep genito-anal in the rabbit, and in addition causes contraction of the body of the penis.

The *scrotal nerves* cause strong contraction of the tunica dartos of the scrotum on the same side.

Origin of the nerve fibres for the genital striated muscle.

Cat. The external sphincter of the vagina is, so far as we have observed, supplied with motor fibres by the same nerves as supply the external sphincter of the anus. This was the case in Exps. 6, 2, 5, 45, 13, 28, 26, 29 (cp. Part I. p. 95). Broadly speaking the same holds for the striated muscles of the penis. It was the case in Exps. 1, 32, 40, 4, 50 (cp. Part I. p. 95). The correspondence in the origin of the motor fibres for the external anal sphincter and for the striated genital muscles has obtained also in later experiments. The only one we need quote is that given below in which the nerves were markedly anterior, and the 2nd sacral nerve instead of causing strong contraction of the external sphincter of the anus and the muscles of the penis had a very slight effect.

Protocol V. *Cat* (*No.* 59). *Effects of stimulation of sacral nerves. Nerves markedly anterior.* Chloroform, later A. C. E. mixture. Expose spinal

cord from the 7th lumbar vertebra downwards; cut it at the upper level of the vertebra. Tie on each side a little centrally of the posterior root ganglion the VIIth lumbar, I, II, III sacral and I, II coccygeal nerves. Isolate them up to their points of exit. Small pieces of sponge were placed in the canal between each pair of nerves, these were removed on both sides of a nerve before stimulating it. For stimulation, a tetanising current was used; the shocks were distinctly felt on the tip of the tongue, but not strongly, with the secondary coil at 10 cm. of scale (= c. at 10). Stimulate the nerves in succession close to the ligature; c. at 10; the nerve on each side was stimulated several times. The results were:

VII Lumbar. Strong contraction of leg, toes wide apart, and claws out. If the electrodes are allowed to touch the cut edge of the bone, or even if passed down the nerve close to its exit, there is also contraction of the sphincter of the anus; but with careful isolation, the nerve being stimulated near the ligature, there is no effect on the external anal sphincter, or on any of the genital muscles.

I Sacral. Strong contraction of leg and foot, but toes not separated nor claws put out. Very strong contraction of external anal sphincter. The scrotum is forcibly drawn up towards the anus. Strong protrusion of the penis, which becomes more flushed at tip. Prepuce is drawn back.

II Sacral. Toes bent. Slight to moderate contraction of external anal sphincter with the left nerve, very slight with the right nerve. Scrotum not drawn up. Penis not protruded, but is seen to swell when prepuce is held back. Micturition, sometimes after, sometimes during stimulation. Some defæcation.

III Sacral. Movement of tail. No effect on external anal sphincter, elevator of scrotum or muscles of penis, and no certain effect (prepuce being held back) on penis itself. Some micturition with earlier stimulations.

I Coccygeal. No effect, except movement of tail.

Use now a stronger current (c. = 8) and stimulate the nerves near their *exit from the bone*. The prepuce was held back by small clamps. The results were (omitting muscular effects described above):

I Coccygeal. } No effect seen in penis or tunica dartos of scrotum. Good
III Sacral. } erection of hair in the region supplied by the nerve stimulated.

II Sacral. Good retraction of penis; and contraction of tunica dartos on the stimulated side.

I Sacral. Sometimes slight contraction of tunica dartos.

The prepuce was cut through, and the body of the penis freed from the surrounding tissue. (c. = 9).

VII Lumbar. No effect on penis.

I Sacral. (Before 1st stimulation, penis retracted.) Strong contraction of ejaculator urinæ; penis swells gradually but greatly, and remains so. On stimulating again, there is quick strong extension and straightening of penis.

II Sacral. Penis quick movement, extends and becomes straighter, movement is slight compared with that produced by I sacral, penis swells; when the electrodes are moved towards the exit of the nerves, there is retraction and curving of the penis.

Expose spinal cord in lower lumbar region. Tie and cut on each side IV, V, VI lumbar nerves. Remove the cord in this region. I Sacral, erection; then stimulate IV lumbar, the penis contracts, is bent dorsally by the retractor muscle, then shrinks further and becomes flaccid; II Sacral, erection, then stimulate Vth lumbar, no effect; then IVth lumbar, there is very strong retraction of penis; stimulate III sacral, no effect seen, and no effect with VIth or VIIth lumbar.

Cut through abdominal wall in linea alba to expose the bladder. Inject into jugular vein 2 c.c. 1 p.c. curari, in order to paralyse the striated genital muscles.

I Sacral. Slight to moderate contraction of bladder, strong swelling of penis.

IV Lumbar. Slight to moderate contraction of bladder, great shrinking of penis, contraction of vas deferens and of tunica dartos of scrotum.

II Sacral. Strong contraction of bladder, strong swelling of penis.

III Sacral. Fairly strong contraction of bladder; no certain effect on penis.

V Lumbar. No effect on bladder or penis.

Inject into jugular, 25 mgs. nicotin. After this no nerve caused contraction of the bladder or swelling of the penis. The IInd sacral nerve stimulated near its exit caused retraction of the penis and contraction of the tunica dartos.

The scheme of innervation of the striated genital muscles of the cat, both male and female, is then

	Markedly Anterior		Moderately Anterior, Median and Posterior (*a*)	Posterior (*b*)
	i	ii		
VII	0	0	0	0
I	**G**	**G**	**G**.G[1]	G
II	S	M	G	**G**
III	0	0	0	0

[1] In some instances of Median and Posterior (*a*) Class, we have not noticed that the 1st sacral gave a stronger effect than the 2nd sacral nerve, but we have not worked the matter out.

So far as regards the nerves which are effective, our results agree with those of Sherrington on the external sphincter of the vagina. Sherrington treats somewhat broadly the variations in origin which correspond with variations in the arrangement of the nerves. He states that in his experiments the 2nd sacral gave usually a greater effect than the 1st, on the vaginal sphincter, *i.e.* presumably in the whole post-fixed class. In the summary of the characteristics of the pre-fixed class (*op. cit.* p. 640) he gives the 2nd sacral as having a slight effect only. Both of these appear to us to be extreme variations, of the former we have observed one instance only, and of the latter but two.

The several striated muscles of the genito-anal region are not, however, innervated in exactly the same way. If they were the complex movement produced by the 2nd sacral nerve should be the same as that produced by the 1st sacral, except in strength. But this is rarely, if ever, the case. In all cases, when we have noticed carefully the character of the movements produced by the two nerves, there were some differences. Thus when the penis is freed from surrounding tissue up to the limb-muscles, the 1st sacral may cause chiefly a ventral movement of the penis, whilst the 2nd sacral may cause chiefly an outward, straightening movement. Nevertheless, the only muscle which we have definitely found to be innervated by a single spinal nerve is the elevator of the scrotum. And this only when the arrangement of the nerves is markedly anterior (cp. Exp. above).

In the rabbit, also, we have found no certain difference[1] between the origin of the nerves for the striated genital muscles and that of the nerves for the external anal sphincter. A correspondence in their origin was observed in the experiments given in Part I. p. 92. In male animals the protrusion of the penis, in female animals the contraction of the external vaginal sphincter, was observed at the same time as the contraction of the external sphincter of the anus. Since writing Part I. we have made further experiments, and with similar results. One of these we may mention, since the arrangement of nerves was more posterior than any we had met with previously. In this (No. 34), the 2nd sacral nerve gave a slight contraction only, the 3rd and the 4th sacral both gave a strong contraction.

Taking all our experiments into consideration, we deduce the

[1] In Exp. No. 28, in which the 4th sacral nerve caused slight contraction of the external sphincter, it did not cause any jerking movement of the penis, but as the genital muscles were not exposed they may have contracted slightly.

following scheme for the innervation in the rabbit of the striated muscles of the external generative organs.

	Anterior	Median to Posterior	Posterior (α)	Posterior (β)
I	0	0	0	0
II	G	G	G	S
III	G	G	G	G
IV	0	0	S	G
I	0	0	0	0

It will be noticed that there is greater variation in the innervation of the somatic genital muscles in the rabbit than there is in the cat.

In the rabbit as in the cat, the movement of the penis produced by the several nerves varies somewhat in character, and we think it possible that, in both, particular muscles are innervated by a single nerve.

Origin of visceral erector fibres.

On the origin of the inhibitory fibres to the external generative organs, we have made a large number of experiments. The results were the same in the male and the female, so that we may treat these together. Most of the experiments were made by stimulating with weak and with strong tetanising currents the several sacral nerves, and observing the various results produced. One point especially must be noticed. The degree of protrusion of the penis caused by a particular spinal nerve is no satisfactory indication of the number of vaso-dilator fibres it contains. Protrusion of the penis may be produced by the action of the striated muscles, independently of any coincident action of vaso-dilator fibres. All that is necessary for this, is that the penis be moderately distended with blood. Considerable and even great distension may be produced by section of the lumbar sympathetics. In such case great protrusion may be obtained by stimulating the pudic nerves which contain no dilator but only constrictor fibres. The protrusion, of course, is transient, the vaso-constrictor and retractor fibres soon coming into action. In the case however of the spinal nerves, the protrusion may continue, provided the stimulus be applied to the nerve in such a way as to avoid stimulation of the sympathetic fibres. For convenience the protrusion of the penis caused by the action of its striated muscles may be spoken of as somatic erection, and the enlargement of the penis produced by inhibitory fibres as visceral erection.

In both rabbit and cat, the lowest effective nerve nearly always produces pure visceral erection. With a markedly posterior arrangement of nerves, the highest effective nerve has once or twice appeared to produce pure somatic erection, but we have not been certain of entire absence of visceral effects.

The effect of the striated muscles can of course be done away with by giving curari. But on stimulating the spinal nerve in the most convenient place, *i.e.* peripherally of the ganglion of the posterior root, there still remains in most cases the conflicting action of the proper inhibitory fibres of the nerve and the motor fibres of the grey ramus joining it. With care the nerves which contain inhibitory fibres can nevertheless be determined, but it is certainly more satisfactory to cut the pudic nerves on one side at least before testing the spinal nerves for inhibitory fibres. Since, however, section of the pudic nerves, by cutting off constrictor impulses, may cause considerable distension of the penis, it may be advisable to stimulate the peripheral end of one of the pudic nerves in order to obtain constriction, and then to observe the effect of stimulating the spinal nerve.

The results we have come to regarding the origin of the inhibitory fibres, deduced from six experiments in which the pudics were cut and in about forty in which they were uncut, are as follows:

	Cat			Rabbit		
	Anterior	Median	Posterior (*a*)	Anterior	Median	Posterior
VII	0	0	0	0	0	0
I	G	G	M	0	0	0
II	G	G	G	G	S	?S
III	?0	S	S.M	G	G	G
IV	0	0	0	S	S.M	G
V	0	0	0	0	0	?0

In one or two experiments made on the dog, the origin of the inhibitory nerve fibres was the same as in the cat.

All the inhibitory effects of the sacral nerves are produced by fibres which pass out in the pelvic visceral branches (nervi erigentes). For (1) stimulation of the peripheral end of the cut pelvic nerves causes all the inhibitory effects we have mentioned above; and (2) if the nervi erigentes be cut, stimulation of the sacral nerves no longer has any inhibitory effect. The effects, if there are any, are motor.

SUMMARY.

The upper—or lumbar—set of nerves cause, in the male cat, strong contraction of the arteries of the penis, slight contraction of the arteries of the surrounding genital skin and of the scrotum, strong contraction of the retractor muscle, and of the other unstriated muscle of the penis. These effects combine to produce considerable retraction of the organ. The nerves cause also strong contraction of the tunica dartos of the scrotum, and of the unstriated muscles of the prepuce; and contraction leading to slight movement in the surrounding skin.

In the male rabbit, the effects are the same except that there is very slight contraction in the scrotal skin, and there is marked contraction in the skin over the body of the penis.

In the dog, the effects are as in the cat, except that externally very little effect is seen. But the retractor muscle is readily exposed and its contraction is then conspicuous.

In the female, the effects are, *mutatis mutandis*, the same as in the male. But as a rule, they are less marked, and this is especially the case in the cat and dog with regard to the effects which are not vascular. In the rabbit, the movement of the skin covering the vulva is the strongest and most constant effect.

The origin of the nerves from the spinal cord, we take to be the same in the male and female, although the nerves which have a weak effect in the male, not infrequently have no perceptible effect in the female.

In the *cat*, the 13th thoracic and the 1st lumbar nerves sometimes send a few fibres to the genital arteries, causing slight pallor and shrinking, but we have not been certain of any other effect from these nerves.

The 2nd lumbar has as a rule a slight but quite distinct action on the external generative organs.

With an anterior arrangement of nerves, the 3rd and 4th lumbar have a strong action, and the 5th lumbar no action.

With a posterior arrangement of nerves, the 3rd lumbar has a slight to moderate action, and the 4th and 5th lumbar a strong effect.

In some cases, the nerves which cause contraction of the scrotum are as a whole anterior to those which have a distinct effect on the penis.

We have not worked out in detail the variations in the effect of the several nerves which may correspond with the variations in the forms of

the lumbo-sacral plexus, but judging from the experiments in which we have paid most attention to the matter they are as follows:

	Anterior	Median	Posterior (*a*)	Posterior (*b*)
II L.	S	S	?S	?S or 0
III L.	G	G	M	S. M
IV L.	G	G	G	G
V L.	0	0 or S	M	G
VI L.	0	0	0	0

In the *dog*, the innervation differs in two or three respects from that in the cat. The 5th lumbar does not send fibres to the external generative organs, even with a posterior arrangement of nerves; nor the 4th lumbar with an anterior arrangement. The 1st lumbar has a distinct action, and the same is the case with the 13th thoracic, and even with the 12th thoracic. The nerve fibres to the scrotum are more limited in origin than those to the penis; they arise from the 1st to the 3rd, or from the 2nd to the 4th lumbar nerves.

In the *rabbit*, we have found no effect from the 1st lumbar nerve (three) experiments, and twice only from the 2nd.

The 3rd, 4th and 5th lumbar nerves have always an effect, more or less pronounced.

The fibres from the lumbar nerves run in the white rami communicantes, to the sympathetic chain. From this there are two paths, the one (*a*) by the pudic nerves, the other (*b*) by the pelvic plexus.

(*a*) The great majority of the fibres take the course of the pudic nerves. They run down the sympathetic chain to the sacral ganglia. From these ganglia, fibres are given off, which run in the grey rami communicantes to the sacral nerves. The fibres run on in the pudic nerves (*i.e.* in somatic branches); sending, apparently, none to the nervi erigentes (*i.e.* to the visceral branches). The several sacral nerves take a different share in the formation of the pudic nerves in different individuals; and the effect which the several grey rami have on the generative organs varies in correspondence. The scheme of these variations is given on p. 112.

The pudic nerves vary only in origin, their peripheral distribution is the same in all animals of one species. The effects of stimulating the several peripheral branches is given on p. 113. This shows also the branches by which the sacral grey rami, *i.e.* the fibres from the lumbar nerves, make their way to the periphery.

(*b*) The second course, viz. that by the pelvic plexus, has compara-

tively few fibres. Most of them run in the hypogastric nerves. A few may join the plexus from the lower lumbar or upper sacral sympathetic chain, or from the aortic plexus. And of these some may join the first root of the nervus erigens, and run with it to the pelvic plexus.

The cremaster is absent in the rabbit and cat. It is present in the dog. Its motor fibres reach it by the genital branch of the genito-crural nerve, they arise from the 3rd or 4th lumbar nerves according as the plexus is anterior or posterior.

In the upper set of nerves we have found no satisfactory evidence of the presence of vaso-dilator fibres for the blood vessels, nor of inhibitory fibres for any unstriated muscle of the genital organs or genital skin.

The lower or sacral set of nerves cause, as is well known, contraction of certain striated genital muscles, dilation of the arteries of the penis (or vulva). They cause also inhibition of the unstriated muscle or body of the penis, the retractor muscle of the penis, of the vulvar muscles. The retractor muscle appears to us to offer advantages for the study of inhibition of unstriated muscular tissue, presented by no other muscle in which inhibition is known to occur. The sacral nerves cause also in the rabbit inhibition of the unstriated muscles of the genito-anal region.

The scheme of innervation of the striated muscles is given on pp. 115 and 117. That for the visceral inhibitory fibres on p. 118. The sacral nerves—so far as we have been able to determine—send no visceral fibres by their somatic branches.

When we compare the origin from the spinal cord of the nerves for the external generative organs with that of the nerves for the end of the intestine and for the bladder, we see that there is no essential difference as regards the lumbar nerves. The difference consists in the nerves near the junction of the thoracic and lumbar regions sending efferent fibres to the external generative organs more frequently than they do to the end of the intestine, just as these nerves more frequently send fibres to the end of the intestine than they do to the bladder.

With the sacral nerves however the matter is different. The origin of the inhibitory nerves which run to the external generative organs is more anterior than the origin of the nerves which run to the end of the intestine and to the bladder; the areas of origin overlap to a large extent, but the one extends a little farther towards the head, and the other a little farther towards the tail.

PART IV. THE INTERNAL GENERATIVE ORGANS.

By the internal generative organs we mean those which are not developed from the uro-genital sinus or from the surrounding skin. But to some of these we have paid very little attention. Thus, we have examined the testis and ovary in a few cases only, and then solely for vascular changes. The special parts observed, we shall mention below in the account of the results obtained in each animal.

Previous Observations[1]. The experiments of Valentin, Brachet, Longet, Budge, and others showed that contraction of the uterus could be obtained by stimulating the spinal cord—especially in the upper lumbar region—and by stimulating certain parts of the sympathetic system. Budge obtained contraction of the vasa deferentia in the rabbit by stimulating the spinal cord at the level of the 4th lumbar vertebra, and also by stimulating a certain sympathetic ganglion,—apparently the inferior mesenteric ganglion. Later Loeb obtained contraction of the vasa deferentia and prostate vesicles from the hypogastric nerves. Frankenhaüser, Körner and others found that

[1] Some account of the observations made before 1852 is given by Röhrig (quoted below). Compare also S. Meyer, Hermann's *Handbuch d. Physiol.* Bd. v. Th. 2. L. 1, p. 467. 1881.

Kilian. *Zeitsch. f. rat. Med.* N. F. II. 1. 1852.
Budge. *Virchow's Archiv*, XV. 115. 1858.
Spiegelberg. *Zeitsch. f. rat. Med.* B. III. Bd. II. 1. 1858.
Frankenhäuser. *Jenaische Zeitsch. f. Med.* I. 35. 1864.
Oberneier. *Experim. Unters. ü. d. Nerven des Uterus.* (Diss. Bonn.) 1865.
Körner. *Studien d. physiol. Instituts zu Breslau.* Hf. 3. 1865.
Loeb. *Beiträge zur Bewegung d. Sammleiter.* (Diss. Giessen.) 1866.
Frankenhäuser. *Die Nerven der Gebärmutter.* (Jena), 1867.
Oser u. Schlesinger. *Wiener med. Jahrbücher.* 1872. p. 57.
Schlesinger. *Wiener med. Jahrbücher.* 1873. p. 1.
Goltz u. Freusberg. *Pflüger's Archiv*, IX. 552. 1874.
v. Basch u. Hofmann. *Wiener med. Jahrbücher.* p. 465. 1877.
Röhrig. *Virchow's Archiv*, Bd. 76. p. 1. 1879.
Fellner. *Centralb. f. d. med. Wissensch.* p. 258. 1887.
Langley. *Proc. Physiol. Soc.* Dec. 1890. (This *Journal*, XII.)
Sherrington. This *Journal*, XIII. 684. 1892.

efferent fibres left the lumbar region of the spinal cord, and passed to the uterus by way of the sympathetic, the inferior mesenteric ganglia, and the aortic plexus. As to the origin of these fibres from the spinal cord, Langley found that most of them, both from the uterus and vasa deferentia, traversed the sympathetic in the region from the 4th to the 6th lumbar ganglion, so that presumably most of them arose from the 3rd, 4th and 5th lumbar nerves. Sherrington found that the 2nd and 3rd lumbar nerves in Rhesus, and the 3rd and 4th lumbar nerves in the cat, contained motor fibres for the vasa deferentia.

There is much difference of opinion with regard to the effect of the sacral nerves on the uterus. Frankenhaüser at first considered them to have an inhibitory action, later (1867) he withdrew this opinion and left the question open. Kehrer, Körner, and Röhrig state that they obtained contraction of the uterus on stimulation of the sacral nerves. v. Basch and Hofmann, and Fellner hold that the nervus erigens causes contraction of the longitudinal and inhibition of the circular coat of the uterus, and that the hypogastric nerve causes contraction of the circular and inhibition of its longitudinal coat. v. Basch and Hofmann also state that the nervus erigens contains dilator fibres, and the hypogastric nerve constrictor fibres for the uterine blood vessels.

Effects of the Upper Set of Nerves in the Female.

Rabbit. In the rabbit, the parts observed in all cases have been the uterus and the vagina. We made one or two experiments on the Fallopian tubes, they behaved exactly like the uterus. It will be remembered that in the rabbit the vestibule is well developed and is covered by a venous plexus which, when filled, forms an almost continuous and thickish layer of blood over it. The vagina stretches above this for six or more centimetres. It is of a rather opaque white colour, and has no venous sinuses. Its circular muscular coat is thicker than its longitudinal. It has sometimes been called the body of the uterus. There are two uteri joined by tissue for about three-quarters of a centimetre before they open into the vagina; this they do separately. They are sometimes called the cornua of the uterus.

The lumbar nerves have two effects on the uterus and on the vagina; they cause contraction of the small arteries, leading to pallor of the organ, and contraction of the whole musculature. The paling may take place very slowly, but the final state of pallor is nearly always great. The contraction of the muscular coats is as a rule very

strong; in the vagina the contraction can usually be seen to start from one end, and spread to the other; the whole then remains for a time in a state of powerful tonic contraction. The pallor and contraction do not commonly run a parallel course, sometimes pallor is obtained before contraction, and in rare instances there is pallor only. The uterus and the vagina do not always respond equally to nerve stimulation; and in such case either one or the other may be the more sluggish.

Cat. In the cat, the effects are of the same nature as in the rabbit, but they are in most cases less marked. Pallor is constant, but of very varying intensity. Contraction is inconstant, when it occurs, it is occasionally strong, usually it is weak.

Both in the rabbit and cat the contraction of the longitudinal muscular coat, on stimulating the lumbar nerve fibres in any part of their course, can be seen with the eye without difficulty. In favourable cases, the longitudinal shortening is most striking.

The lumbar nerves cause also contraction of the unstriated muscle fibres of the mesometrium, whatever their direction.

Stimulation of the lumbar nerves on one side causes at times slight contraction and pallor of the uterus on the opposite side.

The uterus and vagina become fatigued on frequent stimulation, sooner than any other of the organs we have dealt with; hence the longer the stimulus is applied, the longer should be the interval between the stimulations. Röhrig noted that atropin does not in the rabbit paralyse the motor fibres for the uterus. The maximum amount of atropin given by him is not stated. We have injected up to 50 mgs. into a vein, and so far can confirm his result. And similarly, after this amount of atropin strong contraction of the vasa deferentia and seminal vesicles can still be obtained by stimulating the peripheral ends of the hypogastric nerves.

Effects of the Upper Set of Nerves in the Male.

In the rabbit and cat we have in all cases observed the vasa deferentia, and in nearly all cases the seminal vesicles. The organs which in the rabbit we call for convenience seminal vesicles—as others have done on physiological grounds—are in fact the two-horned uterus masculinus. The uterus masculinus has been called also, for example, by Krause, the prostatic vesicle.

The lumbar nerves cause in both rabbit and cat, strong contraction of the whole musculature of the vasa deferentia and seminal vesicles. Pallor is also caused, but on account of the strong contraction, it is less evident as an independent phenomenon than the pallor of the uterus in the female.

The vas deferens in contracting becomes one to three centimetres shorter; so that here there can be no doubt that the longitudinal coat takes part in the contraction.

The contraction of the seminal vesicles is strong enough to cause emission of semen from the penis; we have mentioned earlier that the contraction of the bladder, which is caused by the lumbar nerves, is not strong enough to cause micturition.

Unilateral stimulation causes unilateral contraction of the vas deferens, and bilateral contraction of the seminal vesicles, beginning on the stimulated side.

In the dog, the contraction of the vas deferens, on stimulation of the upper lumbar nerves, is as a rule not very obvious, and there is little or no longitudinal contraction. In sections of the vas deferens it is seen that the musculature is arranged almost entirely in a circular manner. Thus it appears that in the cat and rabbit the shortening of the vas deferens is brought about by the contraction of its intrinsic unstriated muscle, whilst in the dog, this is effected in its upper (testicular) region only and by the action of the striated cremaster muscle (cp. Part III. p. 92).

Origin from the Spinal Cord of the Lumbar Nerve Fibres.

In the rabbit, we have not noticed that any effect is produced on the uterus, the vagina, the vasa deferentia or seminal vesicles, by stimulating the sympathetic chain above the 3rd lumbar sympathetic ganglia. The sympathetic between the 3rd and the 4th lumbar ganglia usually gives slight contraction and pallor, but sometimes there is no effect. Between the 4th and the 6th lumbar sympathetic ganglia, strong contraction and pallor of the uterus and vagina,—or of the vasa deferentia and seminal vesicles—are constantly obtained. Between the 6th and 7th lumbar ganglia, the sympathetic sometimes has a slight effect, sometimes none. Below the 7th lumbar ganglia, we have not found any effect from stimulation of the sympathetic. From this we may fairly conclude that the 3rd, 4th and 5th lumbar nerves send

efferent fibres to the structures in question, and sometimes the 2nd lumbar nerve also.

We have made a certain number of observations both in cat and rabbit, stimulating the lumbar nerves in the vertebral canal. The results we give in the following Tables.

Origin of Efferent Nerve Fibres in the Rabbit.

	Uterus and Vagina.			Vasa Deferentia and Seminal Vesicles.				
Exp.	9	19	25	27	37	23[1]	24/32	26
I Lumbar	0	—	—	0	0	—	—	—
II „	↑0	—	—	0	0	—	—	—
III „	S	M.G	—	G	S.M	0	—	0
IV „	G	G	G	—	G	G	G	G
V „	G	—	—	—	G	G	—	G
VI „	—	—	—	—	0	0	—	—
Arrangement of Nerves	M	M	P	A	A to M	M to P	P	P

Unless otherwise mentioned both contraction and pallor were observed.

[1] The effect was abolished by cutting the rami to the inferior mesenteric ganglion: though the Vth lumbar still caused some contraction of the bladder.

Origin of Efferent Nerve Fibres in the Cat.

	Uterus.				Vasa Deferentia & Seminal Vesicles.				
Exp.	27[1]	26[2]	9[3]	56	8	59	32	63	64
I Lumbar	0	0	↑0	—	—	—	—	0	—
II „	S	↑S	↑S	—	—	—	—	0.S	0
III „	S.M	S.M	M	—	M	—	—	M	S
IV „	M	M	G	G	—	G	M	G	M
V „	↑0	↑0	—	M	—	0	0	M	S.M
VI „	—	0	—	0	—	0	0	0	0
Arrangement of Nerves	M	P. (*a*)	Post. (*b*)		Ant.		A to M	M	P (*a*)

[1] After cutting the left hypogastric nerve, the spinal nerves on the left side had no effect.

[2] Pallor observed, but no certain contraction. After cutting the branches to the inf. mes. gang. on the left side, the spinal nerves on that side had no effect.

[3] After cutting both sympathetics between the 7th lumbar and 1st sacral ganglia, the spinal nerves produced their normal effect.

Course of the Lumbar Fibres.

Both in cat and rabbit, the nerve fibres run by the white rami communicantes to the sympathetic, and nearly all of them run then to the inferior mesenteric ganglia. Occasionally a few leave the sympathetic below the 6th lumbar ganglion, and apparently run direct to the pelvic plexus, but we have not found any to come off lower than the 7th lumbar ganglion.

From the inferior mesenteric ganglia they proceed by the hypogastric nerves[1].

In the rabbit, most of the fibres run to the lower pair of the inferior mesenteric ganglia, for whilst stimulation of these gives constantly good effects, stimulation of the upper pair has a much less effect and occasionally none. Moreover it sometimes happens that the upper pair have an effect on the uterus but not on the vagina.

The contraction and pallor go hand in hand in so far that if the upper pair of ganglia have the one effect, they have the other also.

On stimulating separately the peripheral branches of the hypogastric in the pelvic plexus, separate contraction and pallor may be obtained of the vagina and of portions of the uterus on the same side. Anatomically these strands of the pelvic plexus appear to proceed to a certain extent from the pelvic nerve. Since, however, the pelvic nerve does not carry efferent fibres to the uterus (cp. below) the contraction of the uterus which is caused by stimulating the strands of the pelvic plexus can only be due to efferent hypogastric fibres. We have obtained from the three main vesico-uterine strands of the plexus, proceeding from the ventral to the dorsal strand, separate contraction of the vagina, of the basal part of the uterus, and of the terminal half to two-thirds of the uterus on the same side.

In no part of their course have the lumbar nerves, in our experiments, shown any inhibitory or vaso-dilator action on the uterus or vagina or on the vasa deferentia and seminal vesicles.

The Sacral Nerves.

We do not find that stimulation of the sacral nerves, or of the pelvic nerve near its origin, has any direct effect on the uterus or vagina in

[1] Sherrington's remark (*op. cit.* p. 685) that the efferent fibres for the vas deferens run in the genito-crural nerve must, we think, be due to a slip of the pen. The hypogastric always has an effect, but we have never found the peripheral end of the genito-crural to produce any. It does however cause contraction of the cremaster in the dog.

the female, or on the vasa deferentia or seminal vesicles in the male. Sometimes however the strong downward movement of the end of the colon which is caused by the sacral nerves may serve as a mechanical stimulus to the vagina or prostate vesicle and so cause contraction.

Having failed repeatedly to obtain contraction from stimulation of the sacral nerves, we paid especial attention to the question of the presence of inhibitory fibres. In the ordinary quiescent state of the organs, there was no trace to be seen of elongation or flabbiness, when the sacral nerves were stimulated, nor were the rhythmic spontaneous contractions which sometimes occur modified in any appreciable manner.

We tried then, in the rabbit, simultaneous stimulation of the lumbar and sacral fibres: varying strengths of current were used for each nerve; and the stimulus was applied sometimes first to the lumbar and sometimes first to the sacral nerve. In no case did we find that the stimulation of the sacral nerves influenced in any way the effect of stimulating the lumbar nerves.

We give the following extract from an experiment to indicate the general nature of the observation.

Protocol VI. *Rabbit. Simultaneous stimulation of lumbar and sacral nerves.* Right vas deferens observed. Separate tetanising apparatus used for the lumbar and sacral nerves. With the secondary coil at 10 c.m., the shocks were very distinct to the tip of the tongue, but were not strong.

The right 5th lumbar nerve, and the 2nd, 3rd and 4th sacral nerves (tied together), were stimulated for about 20 seconds, the interval between successive stimulations being 1 to 1½ minute; the latter stimulated with a rather strong tetanising current (c. = 8) giving very strong contraction of the bladder; the former with a tetanising current diminishing in strength in the four successive stimulations (viz. c. = 10, c. = 12, c. = 15, c. = 16). In each case normal contraction of the vas deferens was produced.

After this, the sacral nerves were stimulated alone for 10 seconds, then the stimulus was thrown into the 5th lumbar nerve, and both lumbar and sacral stimulated for about 20 seconds. There were four successive stimulations, the position of the secondary coil being constant for the sacral nerve (c. = 8), and being shifted away from the primary coil in the successive stimulations (c. = 10, 12, 15, 16) of the lumbar nerves. The sacral nerves were still entirely without effect on the vas deferens. A cannula was then tied in the vas deferens about the level of Poupart's ligament, and salt solution at a slight pressure kept in it. The sacral nerves caused no alteration in the level of the fluid in the cannula, the 5th lumbar nerve at once drove it up.

Since the view that the sacral nerves send efferent fibres to the uterus is widely accepted, and since we were unable to find any physiological evidence of the existence of such fibres, it seemed to us highly desirable to test the view in another way. To this end we cut in the cat the roots of the sacral nerves in the spinal canal, and after five or more days examined the branches of the pelvic plexus for degenerated fibres. The results have completely carried out our view that no efferent fibres pass from the sacral nerves to the internal generative organs. Since, however, the results throw some light on the innervation of several of the pelvic viscera, and since it is desirable to deal also with the question of the distribution of sensory sacral fibres, we shall consider, in a separate section, the results obtained by the degeneration method.

Contraction of the uterus has been observed to follow stimulation of the central end of a number of sensory nerves. Röhrig found a similar effect from stimulating the central end of the nervus erigens. We have not paid much attention to the reflex effects, but we may mention one point in this connexion. We have sometimes found in the rabbit that section of the sacral nerves or of the pelvic nerve on one side stops the rhythmic contraction of the seminal vesicle on that side, and that contraction could then be obtained by stimulating either the central end of the cut pelvic nerve, or the central end of the cut sacral spinal cord. This implies that afferent impulses ascend the pelvic nerve—not necessarily from the vas deferens itself—and tend to cause a reflex contraction through the lumbar nerves.

Summary.

The internal generative organs with which we are concerned are the Fallopian tubes, uterus, and vagina in the female; the vasa deferentia and seminal vesicles (or the uterus masculinus) in the male; structures developed from either the Müllerian or the Wolffian ducts.

The origin from the spinal cord of the efferent fibres which run to each of these structures is the same.

The usual relative effect of stimulating the several lumbar nerves is as follows:—

	Rabbit	Cat	
		Anterior	Posterior
II Lumbar	0 or ↑Slight	Slight	↑Slight
III „	Moderate	Good	Moderate
IV „	Good	Good	Good
V „	Mod. to Good	0	Moderate
VI „	0	0	0

The efferent fibres are motor for the muscular walls, and vaso-constrictor for the small arteries. The effect on the uterus and vagina is more constant in the rabbit than in the cat, though in both it varies with the state of the uterus with regard to parturition. In the cat, pallor of the uterus, with little or no contraction, is not infrequently obtained.

Both in the rabbit and the cat, the motor fibres supply the longitudinal as well as the circular muscular coat. This is at times perfectly clear in the uterus, and is always unmistakable in the vasa deferentia.

In the dog, the contraction of the vasa deferentia produced by stimulating the upper set of nerves, is much less than it is in the cat and rabbit; and there is little or no longitudinal contraction. It may be noted that in the cat and rabbit, in which there is strong longitudinal contraction of the vasa deferentia, there is no cremaster muscle, whilst in the dog, in which the longitudinal contraction of the vasa deferentia is slight or absent, the cremaster is well developed.

Unilateral stimulation gives sometimes slight contraction and pallor of the uterus on the opposite side; the crossed action is much less than in the case of the bladder or of the external generative organs.

The course taken by the efferent fibres is the same as that already described for the lumbar fibres to the bladder, except that no fibres for the internal generative organs leave the sympathetic below the 7th lumbar ganglion.

The sacral nerves send neither motor nor inhibitory fibres to any of the internal generative organs.

PART V. POSITION OF THE NERVE CELLS ON THE COURSE OF THE EFFERENT NERVE FIBRES.

We have seen that there are two sets of nerve fibres to the descending colon, rectum, bladder, and external generative organs; that the origin of these from the spinal cord and their peripheral course have many points in common. Hence instead of considering separately the position of the nerve cells on the course of the fibres to each viscus, we take them here together. The efferent fibres to the internal generative organs we can consider at the same time, although these are contained in the upper set of nerves only.

The Upper Set of Nerve Fibres.

After injection of 10 to 15 milligrams of nicotin into a vein of a rabbit or cat, stimulation of the lumbar nerves in the spinal canal has no effect whatever on the viscera[1]. Hence all the visceral fibres of the lumbar nerves must be connected with nerve cells somewhere in their course. It will be noticed that this holds also for vaso-dilator fibres if such are present. Similarly, stimulation of the sympathetic chain from the 1st to the 6th lumbar ganglion has no effect. Hence the nerve cells are not in this portion of the sympathetic chain, but lie in the more peripheral course of the nerves. In other words, the ganglion cells of the lumbar sympathetic chain (1st to 6th) are not on the direct course of the nerve fibres passing to the descending colon, uterus, bladder, or external generative organs, and the ganglia send no fibres to these viscera. We have seen that from the lumbar sympathetic chain the nerve fibres take two main courses (1) outwards to the inferior mesen-

[1] We may recall that the paralysis produced by a small dose of nicotin rapidly disappears; thus in the rabbit and cat, 10 milligrams of nicotin may cause *complete* paralysis of pre-ganglionic fibres for about a quarter of an hour only. In the dog, complete paralysis is brief even after large doses.

teric ganglia, and (2) downwards in the sympathetic chain. These two courses we will consider separately.

(1) *Course through the mesentery.* The spinal branches to the inferior mesenteric ganglia are, *after injection of nicotin*, as ineffective as the spinal nerves or as the lumbar sympathetic, but on reaching the inferior mesenteric ganglia, all the effects of the upper set of nerves can be obtained, but with varying degrees of strength.

Rabbit. In the rabbit, *stimulation of the ganglia* causes pallor and inhibition of the descending colon—commonly with a brief preliminary contraction—as if no nicotin had been given. Although, no doubt, some of the nerve fibres run through the inferior mesenteric ganglia and end in nerve cells more peripherally, the fibres given off to the descending colon by the ganglia are so numerous as to produce an apparently maximal effect.

The stimulation produces also contraction of the bladder, but it is less than normal, implying that an appreciable number of nerve fibres for the bladder end in cells peripherally of the inferior mesenteric ganglia.

The effect on the uterus and vagina—or vasa deferentia and seminal vesicles—varies considerably in different cases. Usually there is pallor and contraction, though the extent of these varies widely. Sometimes no effect is produced until the hypogastric nerves in some part of their course is stimulated, and this part may be in the peripheral branches close to the uterus. It will be remembered that in the rabbit, nerve cells are often found at short intervals along the course of the hypogastric nerves, where they are joined together, as well as in the peripheral branches. On the whole then we conclude that usually some efferent fibres for the uterus and vagina—and the vasa deferentia and seminal vesicles—end in the inferior mesenteric ganglia, but that many run on to the peripheral nerve cells on the course of the hypogastric nerves; that the number which have the former connection is variable; and that occasionally the efferent fibres all pass by the inferior mesenteric ganglia to join the more peripheral nerve cells.

Further some retraction and pallor may occur in the external generative organs, but it is at best small, and not infrequently we have observed none.

In the rabbit, the upper and lower inferior mesenteric ganglia are often a sufficient distance apart to enable them to be stimulated separately. After nicotin has been given, stimulation of the upper one affects chiefly the descending colon and especially its upper part, it has

a slight effect only on the bladder, it commonly has none on the uterus and vagina; stimulation of the lower one also has an effect on the descending colon but chiefly in its lower part, but it has in addition a marked effect on the bladder and usually some on the uterus and vagina. In fact, the nerve fibres given off by the two ganglia have a somewhat different distribution: both send fibres to the descending colon, but the upper sends more to the upper, and the lower to the lower part; the lower ganglion is the chief source of the fibres for the hypogastric nerves.

After nicotin, then, stimulation of the branches ascending from the ganglia, and of the colonic nerves has its usual effects. The effect of stimulating the hypogastric is diminished on the bladder. It is much more diminished on the lower part of the colon, on the rectum, on the internal and the external generative organs. In some cases, no effect is obtained on the two latter.

Careful local application of warm ·5 to 1 p.c. nicotin to the inferior mesenteric ganglia completes the proof obtained by the injection method, as to the connections of the nerve cells. Care is required in such local application, and caution in interpreting the results, since with a thick ganglion, and with a ganglion having a thick sheath, the nicotin may not penetrate to all the cells, and since an excess of nicotin may annul the irritability of the nerve fibres. When 1 p.c. nicotin is, with due precaution, brushed over the whole surface of the inferior mesenteric ganglia and the nerves in the neighbourhood, peripheral effects can still be obtained by stimulation of the rami to the ganglia. On the intestine the effects are usually very slight, and sometimes we have failed to observe any. On the bladder the effect is obvious, though considerably less than normal. On the uterus and vagina—or vasa deferentia and seminal vesicles—the effects are pronounced though somewhat less than normal.

Cat. In the cat we have made similar experiments both on the effect of injecting nicotin into the blood vessels and of applying it locally to the inferior mesenteric ganglia. The results were essentially the same as in the rabbit, bearing in mind the points in which the cat normally differs from the rabbit. Thus in the cat, the upper set of nerves have frequently no effect on the descending colon except pallor, and the same may be the case with the uterus. Naturally, then, stimulation of the inferior mesenteric ganglia, or of the nerves proceeding from them, may, after nicotin, give pallor of the descending colon and uterus without constant or appreciable contraction. In the

cat, the upper set of nerves normally cause contraction of the internal anal sphincter; after nicotin has been injected, a contraction of the sphincter, though a weaker one, is still obtained by stimulating the hypogastric nerves. Lastly, we may mention that when in the cat, the upper part of the hypogastric nerves has no effect on the vasa deferentia and seminal vesicles—or on the uterus—no effect is, as a rule, produced by stimulating the lower part of these nerves. The effect is only obtained when the electrodes are placed on, or past, one of the peripheral ganglia on the branches of the nerves. This is in harmony with the fact that in the cat the trunk of the hypogastric nerve does not contain nerve cells so commonly as it does in the rabbit.

We have already published some observations[1] made on the cat, with regard to the effect of stimulating the hypogastric nerves after section of the nerves themselves or of the rami to the inferior mesenteric ganglia. The results agree with those obtained by the nicotin method. Degeneration of the nerves running to the inferior mesenteric ganglia diminishes but does not annul the normal action of the colonic and hypogastric nerves proceeding from the ganglia. Section of the hypogastric nerves, time being given for degeneration, annuls all the actions of the peripheral portions of the nerves.

Our conclusions, then, are as follows. The nerve fibres which run from the lumbar nerves to the inferior mesenteric ganglia, run to them direct without having nerve cells on their course.

Nearly all the efferent fibres for the descending colon, whatever their function, end in the cells of the inferior mesenteric ganglia.

In these ganglia end also, a considerable proportion—difficult to estimate with any exactness—of the nerve fibres for the bladder and internal anal sphincter, a smaller variable proportion of the fibres for the internal generative organs; and sometimes a few fibres for the external generative organs.

The rest of the nerve fibres are connected with nerve cells placed more peripherally. Thus a few fibres are connected with nerve cells on the course of the colonic nerves; and a larger number run down the hypogastrics to terminate

[1] Langley and Anderson. This *Journal*, xvi. 410. 1894; xvii. 183. 1894.

in the nerve cells of one or other of the small ganglia, which lie on the branches of the hypogastrics and for the most part near the organs they supply.

(2) *Course by the sacral sympathetic chain.* The other course of the efferent nerves, viz. downwards in the sympathetic chain is taken, as we have seen, chiefly by the nerve fibres to the external generative organs. When, after injection of nicotin, the sympathetic trunk is stimulated more and more posteriorly, the characteristic effects of the upper set of nerves are usually first obtained on stimulating the 1st sacral ganglion in the cat, the 2nd in the rabbit, but occasionally a slight effect may be obtained from the ganglion immediately above these. A stronger effect is obtained from the 2nd and 3rd sacral ganglia in the cat—maximal as a rule from the 2nd; and from the 3rd and 4th sacral and 1st coccygeal in the rabbit—maximal as a rule from the 4th.

Hence then the great majority of the nerve fibres to the external generative organs are connected with nerve cells in the sacral ganglia of the sympathetic chain.

There can be little doubt that the variations which occur in different individuals in the order of effectiveness of the several ganglia are in correspondence with the variation in the arrangement of the sacral nerves. We have found this to be so in a few test cases, but we have not thought it worth while to work the matter out in detail. It must be remembered that in the sacral region the ganglia of two successive nerves may be joined together, so that in such cases the grey rami to the several nerves must be stimulated; otherwise it is sufficient to cut the sympathetic between each two ganglia, and to stimulate the ganglia.

The Lower Set of Nerves.

In considering the effect of nicotin on these nerves we must first put on one side the nerve fibres which belong to the somatic division, and supply the external sphincter of the anus, the bulbo- and ischio-cavernosus muscles and so forth. These nerve fibres have no nerve cells on their course, they resemble ordinary motor nerves to skeletal muscle. The nerve-endings of these nerve fibres are paralysed at about the time the motor nerves to skeletal muscle are paralysed; 15 to 25 milligrams of nicotin are required, *i.e.* 5 to 10 milligrams more than are sufficient to paralyse the connexions of the visceral pre-ganglionic nerves with their nerve cells. Since the movements of the striated muscles tend to

obscure the observation of visceral effects, it is better to give 20 to 25 milligrams of nicotin instead of the smaller dose of 10 to 15 milligrams, or to give curari before nicotin.

Nicotin does not paralyse all the visceral effects which are commonly seen on stimulating the sacral nerves in the spinal canal. It prevents the occurrence of the whole of the effects which the sacral nerves produce through the pelvic plexus, viz. all the effects on the colon, upper part of rectum, and bladder, the inhibition of the internal anal sphincter, of the external generative organs, and of the genito-anal skin muscles. But as we have mentioned earlier (Part III. p. 109) it does not prevent retraction and pallor of the external generative organs, nor contraction of the genito-anal skin muscles. We have shown that this is due to stimulation of the grey rami, joining the nerves from the sacral sympathetic ganglia.

There are one or two other points we may mention here. In the rabbit after nicotin has been given we have not observed any effect on the internal sphincter of the anus, on stimulating the sacral nerves in the spinal canal (cp. Part I. pp. 87, 88). There is however marked contraction of the skin over the end of the rectum. In the cat, we have sometimes observed a slight constriction of the sphincter, and more commonly pallor, apparently due to stimulation of the sacral nerves. We conclude then that few, and sometimes none, of the lumbar fibres for the internal anal sphincter which descend the sympathetic as far as the sacral ganglia (cp. Part I. p. 98) take their further course by the sacral grey rami and the pudic nerves.

The pelvic nerve (nervus erigens) stimulated centrally of its first ganglion, after nicotin injection, has usually no effect; occasionally we have seen a trifling contraction of the bladder, due probably to stimulation of fibres joining the pelvic nerve from the aortic plexus. Stimulation just peripherally of the first ganglion causes weak contraction of the bladder; moderate to strong contraction of the recto-coccygeal muscle and in the rabbit moderate to strong contraction of the rectum also. On stimulating more and more peripherally the several strands of the pelvic plexus, the effects obtained increase in strength, and near each organ a spot can generally be found which gives effects, which are to the eye not greatly less than maximal. The effect of the peripheral branches is least—though it is quite distinct—in the case of the vaso-dilator and inhibitory fibres for the external generative organs; we think this is due partly to the exposure which is unavoidable in dissecting out the branches, and partly to the lowered blood-pressure.

On stimulating the peripheral branches of the pelvic plexus, some ganglia on the course of the hypogastric nerves will naturally be stimulated as well as those on the course of the pelvic nerve fibres. Thus for example contraction of a definite part or of the whole of the uterus may be obtained (cp. Part IV. p. 127).

In the rabbit, the anterior and sometimes the antero-median strand of the pelvic plexus gives contraction of the descending colon, but much less than normal; a stronger but still not maximal contraction can be obtained by stimulating the strand of nerve fibres which ascends in the mesentery ventrally of the hypogastric nerves (cp. Anatomical Part). The central end of the united hypogastric nerves, which normally in the rabbit has an effect on the descending colon, rarely if ever has an effect after nicotin has been given.

On careful local application of nicotin to the first ganglion, whether in rabbit, cat, or dog, the effect of the pelvic nerve on the rectum and recto-coccygeal muscle is decreased to a much greater degree than any other action of the nerve.

Hence then the nerve cells for the several organs are scattered on the course of the nerve strands to them, but most of the nerve cells for any one organ are in ganglia near the organ, thus the first ganglion on the course of the pelvic nerve (nervus erigens), which lies by the side of the rectum, is chiefly for the rectum and recto-coccygeal muscle; the ganglia in the lateral ligament are chiefly for the bladder, the ganglia for the colon are chiefly near the colon, those for the generative organs, close to the urethra or in the organs themselves (cp. later, Anatomical Part).

Effects of large doses of nicotin. The injection of large amounts of nicotin has not given us any constant differences in effects from those produced by small ones. On the whole the motor effects after a large dose have been less marked. On stimulating the hypogastric nerves, the contraction of the bladder was more feeble, the contraction of the uterus or vas deferens was more commonly only obtained by stimulating the peripheral ganglia on the course of the hypogastric, *i.e.* the ganglia of the pelvic plexus; the motor effects on the colon and rectum, on stimulating the inferior mesenteric ganglia, were less commonly seen, so that we were inclined to think that a large dose of nicotin had some paralysing action on motor nerve endings of the post-ganglionic fibres.

But on comparing the details of the several experiments this view

seems to us doubtful[1]. We have obtained in the rabbit strong contraction of the recto-coccygeal muscle from stimulating the posterior branch of the pelvic nerve after 130 milligrams of nicotin; good contraction of the colon from stimulating the sacral colonic nerves and the inferior mesenteric ganglia, after 100 milligrams of nicotin. We have obtained also fair contraction of the vasa deferentia from stimulation of the hypogastric nerves, after injection of 65 milligrams of nicotin. But as the two latter effects, at any rate, have not been obtainable in some cases, after a small dose of nicotin such as 20 milligrams, we think it possible that the observed tendency to enfeeblement of the effects of nerve stimulation is due to normal variations in the responsiveness of the tissues in the several cases, and to variations in the position of the peripheral ganglia. We may mention however that in the rabbit inhibitory effects on the intestine, and vaso-constrictor effects, can be obtained with ease by stimulating the inferior mesenteric ganglia, whatever the amount of nicotin given.

It must be noted, in this connexion, that in most of our experiments, we inject the nicotin immediately after giving anæsthetics, before the nerves are dissected, so that in most cases we do not know exactly the degree of contraction which the nerves are capable of producing before the poisoning. We experiment in this way, partly because nicotin is very apt to cause death if it is given after the intestines have been exposed, and partly because dissection of the nerves may interfere with the circulation, and so lead to a lack of proper distribution of the nicotin when injected.

After large doses of nicotin it is not uncommon to find that stimulation of the sacral nerves, or of the sacral sympathetic ganglia, causes much less retraction of the penis than usual, though it may cause complete pallor. This however appears to be due to the state of retraction into which the penis passes after large doses of nicotin. The more retracted the penis and the less the blood-flow through it, the less obvious will of course be the contractor effects of stimulating the nerves.

The Plexuses of Auerbach and of Meissner. We had hoped that nicotin would enable us to show that some one class of nerves are connected with the nerve cells of the plexuses of Auerbach and Meissner. But this hope failed. For as we have already said, nicotin

[1] Occasionally no effect is obtained for 5 to 10 minutes after the injection of a large dose, but as nicotin throws all visceral nerve cells, and the tissues connected with them, in violent action, the absence of effect is probably due to exhaustion.

in large doses does not paralyse any one of the effects which can be obtained by stimulating the fibres given off by the inferior mesenteric ganglia or by the ganglia of the pelvic plexus. It is true that in most cases, motor effects on the intestine were diminished to a much greater extent than inhibitory or vaso-constrictor effects, but on the other hand the difference was not constant, and it is not unlikely that nicotin should have a slight paralysing action on motor nerve-endings in the intestine.

It is however hardly conceivable that some of the sympathetic nerve fibres should not become connected with the numerous nerve cells which are present in these plexuses, and the observations of Ramon y Cajal, v. Gehuchten, v. Kölliker, and Dogiel show fairly conclusively that such a connexion exists.

We may then, at any rate, conclude that these connexions are of a different nature from those which exist between pre-ganglionic fibres and sympathetic nerve cells; and that the nerve cells of the plexuses of Auerbach and Meissner do not belong to the sympathetic system, but are cells of a different class.

Whenever we have injected pilocarpin after nicotin it has caused strong peristalsis. A small quantity of pilocarpin, *e.g.* 5 milligrams, is sufficient. Direct stimulation also causes ready contraction. In both ways we obtained contraction of the descending colon in the rabbit, after the injection of 130 milligrams of nicotin, although in this particular experiment we failed to obtain any distinct contraction by nerve stimulation.

Action of Curarin. We have made a few experiments with curarin since it has on the superior cervical ganglion an action resembling that of nicotin[1]. The action of curarin differs in several points from that of nicotin; thus curarin paralyses nerve-endings in skeletal muscles more readily than it paralyses the endings of pre-ganglionic fibres in sympathetic nerve cells. We have made four experiments, injecting respectively 20, 40, 100, 110 milligrams of curarin into the jugular. In all its main features the action is the same as that of nicotin though a rather larger amount is required to paralyse the pre-ganglionic fibres. After a large amount, motor effects are perhaps more readily obtained from post-ganglionic fibres, and inhibitory effects perhaps less readily.

[1] Langley and Dickinson. This *Journal*, xi. 516. 1890.

OBSERVATIONS UPON THE INITIAL RATES OF OSMOSIS OF CERTAIN SUBSTANCES IN WATER AND IN FLUIDS CONTAINING ALBUMEN.

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Section I. *Introductory.*

There is no doubt if a solution of a crystalloid in water be placed in a closed vessel and be separated from water by a membrane such as copper ferrocyanide, or certain animal and vegetable membranes, that water passes through the membrane towards the side on which is the solution of the crystalloid until a certain pressure has been reached and that then the passage of the water through the membrane ceases: that during this time, in the absence of dialytic action, no molecules of the crystalloid pass in a contrary direction through the membrane: and that all chemically "indifferent" substances used in equimolecular solution in water have approximately the same "final osmotic pressure." These statements may be regarded as certain. But it is obvious that the laws of osmosis obtained by chemists and physicists cannot be applied to biological, and particularly to physiological and pathological problems until it has been proved that the facts above given, together with those others upon which the laws of osmosis have been founded, all of which are ultimately derived from determinations of the final osmotic pressures, are also true at pressures within the limits possible in the animal body. A moment's consideration will show that such a preliminary investigation is absolutely necessary. The final osmotic pressure of a ·1 per cent. solution of sodium chloride is 580 mm. of mercury or a pressure about four times the pressure in the aorta of a good sized dog. In the animal body, therefore, by reason of the limited power of resistance of the walls of vessels and of cells, the final osmotic pressure can rarely be reached, and then only with extremely dilute solutions, so that if osmosis occur

in life the initial rate of osmotic flow must be far more important to the economy than the final osmotic pressure. If the initial rates of osmosis of substances bear the same ratio to one another as do the final osmotic pressures of those substances—and this *a priori* one would expect to be the case—then justifiable deductions may be made from observations on the final osmotic pressures, or what is practically the same thing (since solutions having the same final osmotic pressure have the same freezing point) from observations on the freezing points, and not otherwise.

Further, since the physicist or chemist in his work upon osmosis uses distilled water as his solvent and fluid on the other side of the membrane, the results he obtains must not be applied unreservedly to biological questions until it has been determined that those results are true when the fluids on the two sides of the membrane are no longer distilled water but contain albumen.

Lastly, since it is quite open to question whether the membranes in the animal body can be fairly represented by such a membrane as copper ferrocyanide, it is necessary before deductions obtained with the use of copper ferrocyanide membranes are adopted in biology, to prove that those deductions are also true when the physical membrane is no longer a simple chemical compound, but when it bears some relationship to those membranes normally found in the body. The relationship may be, and probably is, remote, but it is reasonable to assume for example that peritoneal membrane prepared by drying and removal of fat alone is more closely akin to normal living peritoneal membrane than is copper ferrocyanide.

My general aim therefore has been to investigate the initial rates of osmosis on an artificial scheme carrying different kinds of membrane, and to compare the rates when the fluid used as solvent of the chemical substance and on the other side of the membrane is distilled water, when it is slightly albuminous and when it is highly albuminous. The results obtained are being applied—as far as possible—to the animal body, but it has been thought better not to complicate this physiological portion of the subject with the more purely physical portion, and therefore consideration of the physiological application of the physical facts has been reserved for a future communication.

Section II. *Previous Work.*

By far the greater amount of work upon osmosis has been done with a membrane composed of copper ferrocyanide, but since this work is

essentially of a physical nature and does not bear directly upon biological problems it need not detain us here[1]. Graham[2] used a vertical osmometer and as his membrane, ox-bladder, and muslin coated with several layers of egg albumin, which was afterwards coagulated by exposure to steam. He considered it very possible that osmosis occurs in the animal body since he found that sodium chloride, acids and alkalies, all of which are present in the animal, favour the occurrence of osmosis.

From a biological point of view there is but little literature. De Vries[3] used the cells of the leaves of Curcuma rubricaulis, Begonia manicata, and Tradescantia discolor as his membranes; his principal object was the determination of the isotonic values of certain salts.

Hamburger[4] with the same object in view used red blood cells. Wladimiroff[5] investigated the subject of osmosis with certain bacteria. He used six varieties of motile bacteria and ten different salts of the alkalies. He concludes that the greater number of his experiments on osmosis between bacteria and salts agree with the laws of osmosis as laid down by van't Hoff and others. It is obvious however that the motility of micro-organisms may vary in an infinite number of ways besides that, possibly, due to osmosis and therefore Wladimiroff's conclusions are not convincing. Upon the initial rates of osmosis of different substances I have been unable to find any literature whatever.

Section III. *Methods.*

1. *The chemical substances.* The substances used were glucose, sodium chloride and urea, being those substances which are most frequently used in physiological research. They were specially prepared for my purpose by C. A. F. Kahlbaum of Berlin and were certified by him to be "chemically pure." They were always kept in an exsiccator when not actually in use for making up solutions.

2. *The fluids.* The fluids used were distilled water, distilled water containing ·1 per cent. of horse-serum (obtained aseptically) and normal

[1] The physical literature of Osmosis is chiefly to be found in papers by van't Hoff, Arrhenius, Ostwald, Raoult, Guldberg and others in the *Zeitschrift für physikalische Chemie*, from its commencement in 1887.

[2] Graham. *Phil. Trans. Roy. Soc.*, 1854, p. 177.

[3] De Vries. *Pringsheim's Jahrbücher f. Wissensch. Botanik*, XIV. 427. 1884; and *Zeitschr. f. phys. Chemie*, II. 415. 1888.

[4] Hamburger. *Zeit. f. phys. Chem.* VI. 415. 1890; cp. also *du Bois-Reymond's Arch.* (*Physiol. Abth.*), 1887, p. 41. Other papers by Hamburger will be referred to in a later communication.

[5] Wladimiroff. *Ibid.*, VII. 529. 1891.

ox-serum, which, being of a higher specific gravity (1030 or thereabouts) than that of the blood-plasma of the dog (about 1025), and varying in individual samples obtained, was always diluted with the requisite quantity of distilled water to bring it to a specific gravity of 1025. The albuminous solutions were always used as rapidly as possible after being obtained from the animal so as to reduce the possibility of chemical and bacteriological changes to a minimum.

3. *The solutions.* The solutions of glucose, sodium chloride and urea were made by weighing out quantities proportional to the molecular weights of the substances and dissolving in the solvent up to a given volume. In this way error from any contraction or expansion during solution was eliminated. The temperature of the solutions at the time when they were made up was in all cases 15° C. The strength of the solutions used was decinormal.

When the solutions had been made their freezing points were determined by means of a Beckmann's apparatus[1], and they were titrated, in the case of glucose with Fehling's solution, in the case of urea by the sodium hypobromite method, and in the case of sodium chloride with decinormal silver nitrate solution. In this way it was conclusively shown that the substances were chemically pure, and that the solutions to be used were strictly decinormal and equimolecular. It need hardly be noted that the final osmotic pressures of the three solutions is not the same, since sodium chloride is an electrolyte and has a final osmotic pressure—owing to dissociation—nearly double that of either of the other two. The solutions of glucose and urea however had exactly the same freezing point (−0·189° C.) and therefore were isotonic besides being equimolecular.

4. *The membrane.* A considerable number of different kinds of membrane was used in an endeavour to obtain a suitable material, amongst them were gutta-percha tissue, peritoneal membrane of calf coated with dissolved india-rubber or with celloidin, gold beaters' skin, a thin film of celloidin made by dissolving the material in ether and pouring out in a thin layer on a plate of glass, muslin soaked in celloidin solution from which the ether was allowed to evaporate, human amnion, and copper ferrocyanide, but from one or other reason, the principal being that in spite of all precautions they allowed of leakage (except in the case of the copper ferrocyanide cells), they were discarded, and in the later experiments simple prepared peritoneal membrane of calf was in-

[1] This apparatus was used in all determinations of freezing points during the research.

variably used. This membrane, if care be taken in the selection of a piece, is almost impervious, so that air will not pass through excepting under considerable pressure. It nevertheless allows of filtration. Thus, if a thistle-funnel provided with a long stem of narrow bore be filled with water and inverted, after a piece of the membrane we are considering has been carefully tied over the mouth of a funnel, the membrane will be exposed to the pressure exerted by the column of fluid in the stem. After a time very small drops of water are to be seen on the under-surface of the membrane which have obviously passed through it by filtration. In an actual experiment the height of the fluid in the stem of the funnel which, at the commencement was 65 mm., fell as the result of filtration and of stretching of the membrane to 47 mm. during a period of 18 hours. After the adoption of the horizontal osmometer to be immediately described, this small amount of filtration could be neglected, and in all the experiments given in the body of the paper, excepting those in which it is definitely noted that copper ferrocyanide was used, the membrane employed was prepared peritoneal membrane of calf.

5. *The osmometer.* The instrument which carried the membrane was first of all used in a vertical position, but later, on account of the extreme difficulty in avoiding filtration when the experiment extended over a number of hours, the vertical position was abandoned, and all later experiments—and upon these alone the results given in the present paper depend—were made with the osmometer in a horizontal position. The adoption of this position has the further advantages that neither the rate of flow nor the tension of the membrane is modified by hydrostatic pressure. The construction of the osmometer is shown in the accompanying figure.

The osmometer consists of two parts, the reservoir and the funnel, both of which are made of glass. The reservoir is capable of holding 100 c.c. and at one end terminates in a tube provided with a glass tap. The other end is open and cemented to a strong brass flange. There is a small hole (A) at the highest point of the upper surface. About the middle of the reservoir is placed a perforated thin brass diaphragm, the holes in which are 4 mm. in diameter and as many are bored in the plate as possible; a flange is turned down on the plate itself to give it strength and to enable it to be cemented to the reservoir. It lies strictly vertically in the reservoir and its face is strictly plane. The funnel is also made of glass and is provided with a side-piece into which a glass tap is ground. It is capable of containing 11·75 c.c. when filled

to the zero-point on the scale. The mouth is ground plane and over it is fitted a brass plate perforated at the same time as the disc in the

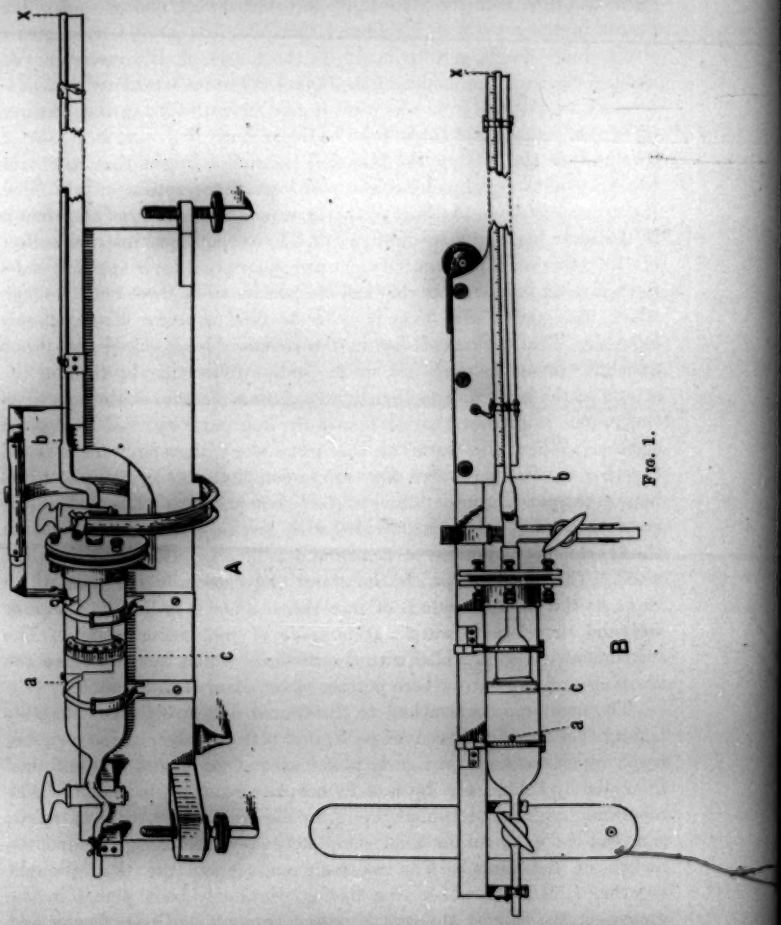

FIG. 1.

reservoir. This plate is very slightly convex forwards and the edges are turned down over the projecting lip of the mouth of the funnel, and

cemented to the glass. The stem of the funnel for 10 cm. consists of glass tubing with an internal bore of about 8 mm., into the side of which opens the tube with the glass tap before mentioned, and around which, nearer to the mouth of the funnel than the side-piece, is cemented a strong brass flange corresponding to the flange on the reservoir. At about 8 cm. from the mouth of the funnel this stem is bent twice so as to form a knee (B), and from this point it goes onwards for a further distance of 45 cm. as a thermometric tube having a bore of 1 mm. in diameter. The knee in the stem of the funnel is of such a height that when the two parts of the osmometer are in position and the apparatus is levelled, the upper surface of the bore of the thermometric portion of the stem is in the same horizontal straight line (ABX) as the upper internal surface of the reservoir. The brass flanges are grooved on their opposing sides to receive an india-rubber ring and are perforated by three holes through which pass screws with nuts in order to bind the two flanges closely together. The perforated disc in the reservoir is so placed that when moderate pressure is applied to the india-rubber ring by turning the screws in the brass flanges the slightly convex plate over the mouth of the funnel is pressed against it and the convexity reduced to a plane surface. When the parts of the instrument therefore are screwed together the two perforated discs are in complete contact, are plane, and have corresponding perforations opposite one another. The instrument rests on a stand which is provided with levelling screws, and beneath the thermometric portion of the stem a millimetre scale is let into the wood. The wood of which the stand and especially the part which supports the stem is made is of importance since it is liable to become wet and therefore to warp. It is made of well-seasoned pine. The instrument is also provided with a spirit-level which can rest upon the reservoir and the narrow bore portion of the stem of the funnel[1].

The membrane is attached to the funnel wet, stretched moderately tightly over the slightly convex perforated plate and over an india-rubber band, which has been previously placed around the mouth of the funnel immediately behind the lip and in no place reaching beyond it. The membrane is secured behind the lip by means of a few turns of waxed silk, and the india-rubber band completely prevents leakage around the mouth of the funnel. The reservoir and funnel are then brought together (the india-rubber ring having previously been placed in the groove on the flange), the screws passed through the brass flanges and

[1] The instrument was made for me by the Cambridge Scientific Instrument Co., and was shown before the Physiological Society in November 1895.

tightened until the brass discs closely fix the membrane between them. The essential points therefore in the apparatus are, *a*, the perforation in the upper surface of the reservoir, *b*, the knee in the stem, by which means the levels of the fluids on the two sides of the membrane when the instrument is filled, are the same, and *c*, the perforated brass plates, which secure as even a tension as possible of the membrane during the course of an experiment.

The method of cleaning the instrument and particularly the thermometric bore is of some importance, since unless the bore be perfectly free from grease and other impurities, the fluid will not run evenly and error is likely to be introduced. By reason of the cement used in the construction and the presence of the metal discs, none of the usual cleansing agents for glass, such as ether, alcohol, strong acids and alkalis could be employed, and it was only after the instrument had been actually put together that I discovered it is impossible to obtain the inside of the glass properly clean unless the cleansing be carried out before the parts are cemented together. The instrument was therefore taken to pieces and the glass thoroughly cleaned from all grease &c., by repeated washings with 10 p.c. caustic potash. It was then well washed with distilled water, dried and cemented together, great care being taken not to soil the inside with grease in any way during the process. The thermometric bore, which is after all the most important part to have *absolutely* free from any contamination, was repeatedly cleansed by filling with 10 p.c. caustic potash, and leaving all night in the horizontal position, the alkali next day being completely removed by washings with distilled water.

After the membrane had been attached and the instrument put together, distilled water was slowly forced into the funnel through the side-piece by means of a syringe until it reached a certain point in the thermometric tube which was noted, the instrument was then left for a certain length of time in the vertical position, and if no obvious leakage occurred through or around the membrane, the reservoir was filled and the membrane was left to soak for about 12 hours. The funnel was then emptied, washed out with some of the solution the initial rate of osmosis of which it was desired to estimate, again emptied and definitively filled with the solution until funnel and stem were completely full. It was of course necessary to hold the instrument vertically for this purpose. The osmometer was then placed on the stand, and the reservoir filled with the fluid used as solvent for the crystalloid under investigation until only a small space was left about the opening at the

top. The fluid in the stem of the funnel was then brought back to the zero point and the experiment commenced. Observations were taken every five minutes and the instrument was left undisturbed during the whole course of an investigation. At the end of three hours, reservoir and funnel fluids were emptied into stoppered bottles and examined severally as to freezing point and percentage composition. In this way it was possible to determine the amount of the substance that had dialysed and also the partial osmotic tension of the system.

After the instrument had been washed twice with distilled water and emptied, it was again washed with a small quantity of the next solution of which the rate of osmosis was to be determined, emptied and then definitely filled as before. The substances were not investigated in any definite order[1], but on one occasion urea was investigated first, on another glucose, on another sodium chloride, while sodium chloride was made sometimes to precede glucose or urea, sometimes to come after it and so on. The object of this was to eliminate the possibility that the results derived from the examination of any one substance really depended upon a modification of the membrane produced by another substance with which it had been in contact during the preceding three hours. It was found that the order in which the substances are investigated does not materially affect the rates of osmosis beyond the fact that, after a membrane has been in use for some days, there is a general diminution of the rates of osmosis of all the substances; thus in the course of three days, during which the same membrane was in use, the initial rates of osmosis of each of the substances was at the end of the time less by about a quarter than it was at the beginning. Nevertheless the ratio between the rates of osmosis of the three substances is not sensibly altered whether the membrane have been a short or a long time in use.

It has already been pointed out that in order to obviate error and obtain readings which are fairly constant during each period of five minutes, considerable attention must be paid to the freedom of the thermometric stem of the instrument from grease or other impurities, but further, the fluid does not run evenly in the bore of the stem if that bore be dry on account of the increased friction which has to be overcome. Although one would expect that such drying would be unlikely to occur, it was nevertheless practically found that it very readily occurs, especially if a gas-flame

[1] The watery solutions were always investigated before the faintly albuminous and these before the highly albuminous solutions. As mentioned later after these had been investigated a control experiment with one of the watery solutions was always made.

be in proximity to the instrument. This difficulty—and it is a considerable one—was overcome by using a strong light at a distance from the osmometer and focussing the rays by means of a lens, on the stem of the instrument and the millimetre scale beneath it whenever a reading was to be taken, and in addition by causing the stem to terminate in a bottle which contained some water. In this way the stem terminates in a saturated atmosphere and a fine mist is seen to collect in the bore of the tube. This is never so great as to collect into droplets and only increases the reading by a negligible amount. It, however, generally obviates the difficulty referred to. Any local increase of friction, whatever be its cause, may also be overcome by gently blowing with the mouth over the opening in the reservoir immediately before a reading is to be taken. If there be no obstacle to the flow in the stem the fluid will run a short distance along the stem and immediately return to its original position. If on the other hand there be a slight obstacle, the slightly increased pressure in the reservoir brought about by blowing is transmitted to the membrane and thence to the edge of the fluid in the funnel, overcomes the obstacle, and the fluid travels onwards but does not return to its former position. When however the fluid has reached that position which it should have attained had no obstacle existed, then blowing over the hole in the reservoir causes it to run forwards with the increased pressure, but directly that pressure is removed the fluid returns to its newly acquired position of equilibrium. No amount of blowing will now further modify the reading when the fluid in the stem has recoiled. Apparently when such an obstacle exists in the stem it is easier for the water which passes through the membrane by osmosis to cause slight bulging of the membrane, this bulging of the membrane however disappearing when the obstacle has been overcome. Blowing over the hole in the reservoir has not been resortod to in any of the experiments given below, excepting in the one where the rate of osmosis of sodium chloride in ·1 per cent. albuminous solution is detailed. Here it is given to show the absence of any effect upon the general regularity of the flow. Nevertheless in all cases at the end of three hours it was resorted to, in order clearly to determine that the amount of osmosis indicated by the position of the fluid in the stem was correct and not under-estimated. No one who has not had practical experience can appreciate the difficulty there is in cleaning the bore so thoroughly that perfectly regular readings over several periods of three hours are obtained without blowing. This difficulty is naturally greatest when albuminous solutions have been used, and of albuminous solutions those containing

glucose cause the greatest amount of trouble. If by chance a droplet formed in the thermometric bore it was immediately removed by passing a fine capillary glass tube down the stem of the instrument. It could not be allowed to remain *in situ*, partly because of the difficulty it introduces in taking the readings and partly because the elasticity of the air between it and the true edge of the solution leads to error in the readings themselves. Its removal however in the manner indicated does not interfere with the experiment in any way.

The last point which it is necessary to consider before we proceed to a consideration of the actual results obtained is the influence of temperature. It is obvious that the fluid in the reservoir may be neglected in this respect, excepting in so far as it mechanically restrains the stretching of the membrane by any expansion of the fluid in the funnel. So far as the reservoir itself is concerned, since there is an opening on the upper surface, any expansion of the fluid would show itself principally by a slight modification in the level of the fluid, and the only effect upon the membrane would be the slightly increased pressure which that modification of the level of the fluid would bring about. But in the case of the funnel the matter is different, here the water in the reservoir forms an inelastic wall, so that the only direction in which an expansion of the funnel fluid can show itself is along the freely open bore of the stem. The funnel therefore is somewhat after the type of a thermometer, and as such any reading due to osmosis must, strictly speaking, be corrected for any error introduced by variations in temperature. The effect of temperature is not only limited in this way. Osmosis itself is affected by modifications in temperature but the changes in this respect are small compared to those due to actual expansion of the fluid itself. The effect of temperature has been considered by placing the same fluid (water) in funnel and in reservoir and marking the position of the fluid in the stem when the temperature of the water was 15° C. The whole apparatus was then placed in an incubator kept at a constant temperature at 35° C. for 18 hours and the position of the fluid in the stem again noted. Next the apparatus was removed from the incubator and left until it had attained the room temperature (15° C.). The effect was that as the result of an increase in temperature of 20° C. (35° C.—15° C.) the fluid registered a difference of 55 mm. in the stem. Upon this basis one may calculate roughly the maximum error introduced by variations in temperature during the course of an actual experiment. One degree centigrade caused a modification of the fluid represented by a distance in the bore of the stem of 2·75 mm. Now the actual daily range of

temperature in the room was about 3·5° C. from 13·5° C. in the morning to 17° C. at night. The room was artificially warmed and as far as possible kept at a constant temperature, the minimum and maximum being as above given. Assuming therefore that the whole daily range occurred during the three hours of a single experiment—a manifest improbability—and that the temperature of the fluid varied at the same rate as the temperature of the air—a manifest impossibility—the effect of temperature would be in any experiment to introduce an error of less than 10 mm. But since the readings have been taken every five minutes, and the maximum possible error over three hours is but a small proportion in most cases of the total reading observed, it follows that for our purpose the effect of temperature may be neglected and for this reason thermometric readings have not been given in the accompanying tables.

Section IV. *Results.*

The number of experiments made has not been sufficiently large, and the results obtained not sufficiently concordant with membranes other than copper ferrocyanide and peritoneal membrane of calf to warrant their inclusion in this paper. It need only be here stated generally that in the majority of cases the results they have given have agreed in the main with those obtained with the peritoneal membrane, though amniotic membrane offers some anomalies. It has been thought that the simplest manner in which the method and its results can be shown is by giving as far as possible *in extenso* the numerical values obtained for the different substances in the course of a typical experiment.

A. *Copper ferrocyanide membrane.*

Experiments with this membrane have been of two kinds, 1st, those done in the room and subject to the (small) variations of the room-temperature, and 2nd, those carried out from beginning to end in a closed chamber kept automatically at a constant temperature of 36·5° C. The copper ferrocyanide cells were those in use and made at the Chemical Laboratory of the University and were lent me by the kindness of Professor Liveing. The special osmometer above described was of course not used with these cells, but care was taken to keep the same level in the fluids inside the cell and outside. The glass tube cemented to the cell was bent at right angles and connected with pressure-tubing to a piece of thermometer tubing; in this way the fluid entering the cell by osmosis travelled horizontally along the thermometric stem and its

position was read by a millimetre scale placed beneath it and serving at the same time as a support.

At the room-temperature osmosis through a copper ferrocyanide membrane proceeds very slowly and for this reason observations each five minutes are impossible. The total amount of flow in 24 hours is all that can be satisfactorily determined, and from those total amounts an indication can fairly be drawn as to the initial rates of osmosis of the substances. Osmosis was always allowed to occur for one period of 24 hours with each substance before the definitive determination of the rate was made so as to allow osmosis with that particular substance to be fully established before commencing an observation. The amount of dilution of the solution caused in that way is so small as to be negligible.

The results obtained in a typical experiment were as follows:

With Urea solution in the cell the fluid travelled a distance of 24½ mm. in the stem during 24 hours.

With Glucose solution in the cell the fluid travelled a distance of 35 mm. in the stem during 24 hours.

With Sodium chloride solution in the cell the fluid travelled a distance of 51 mm. in the stem during 24 hours.

Here we see that in accordance with the final osmotic pressure the initial rate of osmosis of sodium chloride is greater than that of either glucose or urea, all the substances being in equimolecular solution. The ratios however between the actual final osmotic pressures of the substances and the initial rates of osmosis are not the same, as one would *a priori* expect them to be. The most striking point is that though the glucose and urea solutions are isotonic yet the initial rate of osmosis of urea is considerably less than that of glucose.

When we come to consider experiments made at 36·5° C.[1] we find that the increase of temperature has considerably increased the rate of osmosis.

With Urea solution in the cell, the fluid travelled a distance of 127 mm. in the stem during 24 hours.

With Glucose solution in the cell, the fluid travelled a distance of 172 mm. in the stem during 24 hours.

With Sodium chloride solution in the cell, the fluid travelled a distance of 255 mm. in the stem during 24 hours.

[1] Boiled distilled water (containing therefore no large amount of air in solution) was used for these experiments, and care was taken that solutions and apparatus were at the required temperature before commencing an experiment. All the necessary materials were left in the warm chamber for 12 hours previous to use.

The experiments given however under the two headings cannot be compared except so far as concerns the ratios between the initial rates of osmosis of the three substances, since different cells and different thermometric stems were used for each set of experiments[1]. The ratio however sodium chloride : glucose : urea :: 17 : 12 : 8 holds fairly well in both cases, a further indication that the observed initial rates are approximately correctly taken under both conditions.

B. *Prepared peritoneal membrane of calf.*

By reason of the differences obtained between the rates of osmosis when this membrane is used and when copper ferrocyanide is used, this portion of the research has been repeated many times. In all, ten different specimens of this membrane have been investigated and the conclusions given at the end of the paper have in no single instance been contradicted. The same actual figures have not been obtained in every case nor even the same ratios, but since we are not dealing with absolute but with comparative results, this is of the less consequence. The point upon which I wish to lay stress is that the tables given below are fairly taken and typical of the whole series.

It will be seen that the initial rate of osmosis with glucose is greater than that with sodium chloride and the initial rate of osmosis with sodium chloride greater than that with urea. And this, in spite of the fact that the final osmotic pressures of the glucose and urea solutions are the same and the final osmotic pressure of sodium chloride nearly double that of either of them. Moreover the differences between the rates of osmosis are so great and the rate of flow during each period of five minutes so fairly constant over the whole three hours during which each individual substance was under investigation that the general direction of the results may be taken as certain.

Further it will be noticed that even the small quantity of proteid actually added when a ·1 p.c. solution of serum[2] is used in the place of distilled water, considerably reduces the initial rate of osmosis. Moreover the reduction is not the same throughout. The rate of osmosis of urea which is normally small is reduced to a greater extent in a faintly albuminous solution than the rate of osmosis of either glucose or sodium chloride. We still find however that the initial rate

[1] From experiments using the same cell and stem at 16° and at 36·5° C. it appears that the initial rates of osmosis are roughly speaking doubled at the higher temperature.

[2] This addition of serum caused a very doubtful lowering of freezing point of ·001° C. Silver nitrate gave not the slightest haziness. The only noticeable point was a faint cloudiness due to the precipitation of globulin in the distilled water.

TABLE I. *Glucose.*

Water as solvent and as fluid on the other side of the Membrane				1 p.c. albuminous solution as solvent and as fluid on the other side of the Membrane				Serum (sp. gr. 1025) as solvent and as fluid on the other side of the Membrane			
Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours
1 h. 5′	0			9 h. 50′	0			1 h. 5′	0		
10	7½	7½		55	4½	4½		10	3½	3½	
15	14½	7½		10 h. 0′	9	4½		15	6½	3	
20	21½	7		5	12½	3½		20	9½	3	
25	29	7½		10	22	7½		25	12	2½	
30	36	7		15	26½	4½		30	14½	2½	
35	43	7	7⅛	20	31	4½	5⅛	35	17½	2½	2 11/12
40	50	7		25	35½	4½		40	20½	2½	
45	57	7		30	39	3½		45	23	2½	
50	64	7		35	44	5		50	26	3	
55	71	7		40	47½	3½		55	29	3	
2 h. 0′	77½	6½		45	51½	4		2 h. 0′	32	3	
5	84	6½	6⅚	50	53	1½	3½	5	34½	2½	2⅞
2 h. 5′	84			10 h. 50′	53			2 h. 5′	34½		
10	91	7		55	55	2		10	38	3½	
15	97½	6½		11 h. 0′	62	7		15	40½	2½	
20	104½	7		5	67	5		20	43½	3	
25	110½	6		10	71½	4½		25	46	2½	
30	117½	7		15	76½	4½		30	49½	3½	
35	124	6½	6½	20	80½	4	4 7/12	35	52½	3	2 11/12
40	130½	5½		25	86	5½		40	55½	3½	
45	137½	6½		30	90½	4½		45	58½	3½	
50	144	6½		35	95	4½		50	61½	2½	
55	150½	6½		40	100	5		55	65½	4½	
3 h. 0′	157	6½		45	105½	5½		3 h. 0′	69½	3½	
5	163½	6½	6 7/12	50	109	3½	4½	5	72½	3	3½

3 h. 5′	163½		
10	169¾	6¼	
15	176¼	6½	
20	182¼	6	
25	188¼	5½	
30	195	6¾	
35	201½	6½	6$\frac{7}{12}$
40	207½	6¼	
45	213¾	6¼	
50	217¾	6	
55	223	5¼	
4 h. 0′	228	5	
5	234	6	5$\frac{11}{12}$

Freezing point of Funnel fluid at beginning = −0·189° C
Freezing point of Funnel fluid at end = −0·177° C
Freezing point of Reservoir fluid at beginning = 0° C
Freezing point of Reservoir fluid at end = −0·002° C

Total amount of Glucose in apparatus at beginning = ·21 gm
Amount of Glucose found at end (Funnel) = ·197 gm
Amount of Glucose found at end (Reservoir) = ·021 gm
Percentage composition of Funnel fluid (end) = 1·69 gm
Percentage composition of Reservoir fluid (end) = ·021 gm
Partial osmotic tension of Glucose at end is that amount which leads to freezing point −0·175 C

11 h. 50′	109		
55	114	5	
12 h. 0′	120	6	
5	125	5	
10	128	3	
15	130	2	
20	132	2	3$\frac{4}{5}$
25	135	3	
30	138	3	
35	140	2	
40	141$\frac{3}{4}$	1$\frac{3}{4}$	
45	148	6¼	
50	152½	4½	3$\frac{A}{12}$

Freezing point of Funnel fluid at beginning = −0·189° C
Freezing point of Funnel fluid at end = −0·179° C
Freezing point of Reservoir fluid at beginning = −0·001° C (?)
Freezing point of Reservoir fluid at end = −0·001° C

Total amount of Glucose in apparatus at beginning = ·21 gm
Amount of Glucose found at end (Funnel) = ·196 gm
Amount of Glucose found at end (Reservoir) = ·01 gm
Percentage composition of Funnel fluid (end) = 1·68 gm
Percentage composition of Reservoir fluid (end) = ·009 gm
Partial osmotic tension of Glucose at end is that amount which leads to freezing point −0·178° C

3 h. 5′	72½		
10	76½	3¾	
15	78½	2¼	
20	82	3½	
25	85¾	3¾	
30	89½	3¾	
35	94½	5	3¾
40	98¼	3¾	
45	102	3¾	
50	104½	2½	
55	106¾	2¼	
4 h. 0′	110½	3¾	
5	113½	3¼	3¼

Freezing point of Funnel fluid at beginning = −0·761° C
Freezing point of Funnel fluid at end = −0·748° C
Freezing point of Reservoir fluid at beginning = −0·566° C
Freezing point of Reservoir fluid at end = −0·567° C

Partial osmotic tension of Glucose at end is that amount which leads to freezing point −0·181° C

TABLE II. *Sodium Chloride.*

Water as solvent and as fluid on the other side of the Membrane				1 p.c. albuminous solution as solvent and as fluid on the other side of the Membrane				Serum (sp. gr. 1025) as solvent and as fluid on the other side of the Membrane			
Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours
12 h. 50′	0			11 h. 15′	0			8 h. 0′	0		
55	5½	5½		20	2	2		5	½	½	
1 h. 0′	10½	5½		25	4	2		10	1	½	
5	14½	4½		30	7	3		15	1½	½	
10	18½	3½		35	10	3		20	2	½	
15	22½	4		40	13	3		25	2½	½	
20	26½	3½	4⅛	45	16	3	2½	30	3½	½	7/12
25	30	3½		50	18½	2½		35	4½	½	
30	34½	4½		55	21	2½		40	5	½	
35	37½	3½		12 h. 0′	22½	1½		45	6	1	
40	41½	4		5	27	4½		50	7½	1½	
45	45½	4		10	29	2		55	8½	1½	
50	49½	3½	3⅝	15	32	3	2½	9 h. 0′	10	1½	1 1/12
1 h. 50′	49½			12 h. 15′	32			9 h. 0′	10		
55	53	3½		20	35	3		5	11½	1½	
2 h. 0′	56½	3½		25	38	3		10	12½	1	
5	60	3½		30	40½	2½		15	13½	1½	
10	63½	3½		35	42½	2½		20	14½	1	
15	67	3½		40	44½	2		25	15½	1	
20	70	3	3 1/12	45	47	2½	2½	30	16½	1	1 1/12
25	73½	3½		50	51	2		35	18½	1½	
30	77	3½		55	53	2		40	19½	1½	
35	80	3		1 h. 0′	56	3		45	20½	1	
40	83	3		5	58½	2½		50	21½	½	
45	85½	2½		10	60½	2		55	23½	1	
50	89½	4	3½	15	63	2½	2½	10 h. 0′	23½	1½	1⅛

2 h. 50′	89½		
55	92½	3½	
3 h. 0′	96	3½	
5	98	2	
10	101½	3½	
15	104½	3½	
20	106½	1½	2½
25	110½	3½	
30	114	3½	
35	116½	2½	
40	120	3½	
45	123	3	
50	125½	2½	3¼

Freezing point of Funnel fluid at beginning = −0·351° C

Freezing point of Funnel fluid at end = −0·274° C

Freezing point of Reservoir fluid at beginning = 0° C

Freezing point of Reservoir fluid at end = −0·009° C

Total amount of NaCl in apparatus at beginning = ·068 gm

Amount of NaCl found at end (Funnel) = ·0556 gm

Amount of NaCl found at end (Reservoir) = ·0146 gm

Percentage composition of Funnel fluid (end) = ·476 gm

Percentage composition of Reservoir fluid (end) = ·0146 gm

Partial osmotic tension of NaCl at end is that amount which leads to freezing point −0·265° C

1 h. 15′	63		
20	65½	2½	
25	68	2½	
30	70½	2½	
35	72½	2½	
40	75	2½	
45	77½	2½	2 4/17
50	79½	2½	
55	81½	2	
2 h. 0′	84	2½	
5	86½	2½	
10	89	2½	
15	91½	2½	2⅛

Freezing point of Funnel fluid at beginning = −0·351° C

Freezing point of Funnel fluid at end = −0·277° C

Freezing point of Reservoir fluid at beginning = −0·001° C (?)

Freezing point of Reservoir fluid at end = −0·014° C

Total amount of NaCl in apparatus at beginning = ·068 gm

Amount of NaCl found at end (Funnel) = ·462 gm

Amount of NaCl found at end (Reservoir) = ·0163 gm

Percentage composition of Funnel fluid (end) = ·462 gm

Percentage composition of Reservoir fluid (end) = ·016 gm

Partial osmotic tension of NaCl at end is that amount which leads to freezing point −0·268° C

10 h. 0′	23½		
5	28	4½	
10	31	3	
15	33	2	
20	34½	1½	
25	36½	2	
30	38½	2	2 1/12
35	39½	1	
40	42½	2½	
45	43½	1½	
50	44	½	
55	44½	½	
11 h. 0′	44½	½	11/12

Freezing point of Funnel fluid at beginning = −0·933° C

Freezing point of Funnel fluid at end = −0·860° C

Freezing point of Reservoir fluid at beginning = −0·566° C

Freezing point of Reservoir fluid at end = −0·584° C

Note. By some error ·608 gm NaCl per cent. was added to the serum instead of ·585 gm as was intended and as was the case with the watery and the ·1 % albuminous solutions.

Partial osmotic tension of NaCl at end is that amount which leads to freezing point −0·276° C

TABLE III. *Urea.*

Water as solvent and as fluid on the other side of the Membrane				1 p.c. albuminous solution as solvent and as fluid on the other side of the Membrane				Serum (sp. gr. 1025) as solvent and as fluid on the other side of the Membrane			
Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min	Average rate per 5 min. during half-hours	Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours
9 h. 20′	0			6 h. 35′	0			1 h. 20′	Edge of fluid remained stationary throughout the whole length of the experiment, at end seen by tilting the osmometer that no obstacle was present to impede flow	No movement of column of fluid at all	
25	2½	2½		40	0	0		25			
30	3½	1½		45	1½	1½		30			
35	4½	1		50	1½	0		35			
40	5½	1		55	1½	0		40			
45	6½	1		7 h. 0′	1½	0		45			
50	7½	½	1⅝	5	1½	0	⅛	50			
55	8	½		10	2	1		55			
10 h. 0′	9	1		15	2½	1		2 h. 0′			
5	9½	½		20	2½	0		5			
10	10½	1		25	3	½		10			
15	11½	1		30	3½	½		15			
20	12½	1	1⅛	35	3½	0	⅛	20			
10 h. 20′	12½			7 h. 35′	3½	Amounts too small for accuracy and therefore only total result taken	Amounts too small for accuracy	2 h. 20′			
25	13½	1		40				25			
30	15	1½		45				30			
35	16	1		50				35			
40	17	1		55				40			
45	18	1		8 h. 0′				45			
50	19	1	1⅛	5				50			
55	20	1		10				55			
11 h. 0′	21	1		15				3 h. 0′			
5	22	1		20				5			
10	23	1		25				10			
15	24	1		30				15			
20	24½	½	⅛	35				20			

11 h. 20′	24½			
25	25½	1		
30	26½	1		
35	27½	1		
40	28½	½		
45	29½	1		
50	30	½		½
55	31	1		
12 h. 0′	32	1		
5	33	1		
10	34½	1½		
15	35	½		
20	36	1		1

Freezing point of Funnel fluid at beginning = − 0·189° C
Freezing point of Funnel fluid at end = − 0·154° C
Freezing point of Reservoir fluid at beginning = 0° C
Freezing point of Reservoir fluid at end = − 0·004° C

Total amount of Urea in apparatus at beginning = ·07 gm
Amount of Urea found at end (Funnel) = ·058 gm
Amount of Urea found at end (Reservoir) = ·0126 gm
Percentage composition of Funnel fluid (end) = ·49 gm
Percentage composition of Reservoir fluid (end) = ·013 gm
Partial osmotic tension of Urea at end is that amount which leads to freezing point − 0·15° C

8 h. 35′				
40				
45				
50				
55				
9 h. 0′				
5	ditto	ditto		
10			Average rate for preceding 4 half-hours per 5 min.	
15				
20				
25				
30				
35	10			1½

Freezing point of Funnel fluid at beginning = − 0·189° C
Freezing point of Funnel fluid at end = − 0·159° C
Freezing point of Reservoir fluid at beginning = − 0·001° C (?)
Freezing point of Reservoir fluid at end = − 0·006° C

Total amount of Urea in apparatus at beginning = ·07 gm
Amount of Urea found at end (Funnel) = ·059 gm
Amount of Urea found at end (Reservoir) = ·0117 gm
Percentage composition of Funnel fluid (end) = ·508 gm
Percentage composition of Reservoir fluid (end) = ·0117 gm
Partial osmotic tension of Urea at end is that amount which leads to freezing point − 0·153° C

3 h. 20′			
25			
30			
35			
40			
45			
50	ditto	ditto	
55			
4 h. 0′			
5			
10			
15			
20			

Freezing point of Funnel fluid at beginning = − 0·769° C
Freezing point of Funnel fluid at end = − 0·724° C
Freezing point of Reservoir fluid at beginning = − 0·566° C
Freezing point of Reservoir fluid at end = − 0·570° C

Partial osmotic tension of Urea at end is that amount which leads to freezing point − 0·154° C

of osmosis with glucose is greater than that of sodium chloride, and the initial rate of osmosis with sodium chloride is greater than that with urea.

When we come to consider the substances in strongly albuminous solutions we find the initial rates of osmosis still further reduced. It might be expected from the effect produced by a ·1 per cent. albuminous solution that osmosis would be completely stopped when ordinary serum is used as a solvent and as fluid on the other side of the membrane, but this is not so excepting in the case of urea. With urea it has invariably been found either that no osmotic flow occurs at all or else—in a minority of cases—the flow is in the opposite direction to that in which it might be expected, in other words we have "negative osmosis." This has not been due to any defect in the membrane or difference in the levels of the fluids on the two sides of the membrane for especial attention has been directed to these two points, and since "negative osmosis" is so little understood and the amounts I have found have been very small—not exceeding 7 mm. in 3 hours—I have preferred to regard them as due to experimental error. In any case, however, they do not modify the general result obtained, which again is that the initial rate of osmosis with glucose is greater than that with sodium chloride and the initial rate of osmosis with sodium chloride greater than that with urea, though the rates of osmosis with each substance is considerably smaller than it is when the solvent and fluid on the other side of the membrane are distilled water.

From these results there follows the paradox that if so-called isotonic solutions be placed on the two sides of a peritoneal membrane under conditions such as those we are now considering, osmosis can nevertheless occur. One may go even further and say that osmotic flow may take place from a solution having a higher final osmotic pressure towards a fluid having a lower final osmotic pressure. If, for example, equimolecular solutions of sodium chloride and glucose be separated by peritoneal membrane the osmotic flow will take place *from* the fluid having the higher final osmotic pressure (sodium chloride) instead of towards it as one would naturally expect. Below is given an example in which such an experiment was made. The glucose solution was placed in the reservoir of the osmometer and the sodium chloride solution in the funnel. The fluid was caused to stand in the thermometric stem at some distance from the zero point and if the results obtained in the previous experiments are accurate it follows that the fluid in the stem should run backwards towards the belly of the funnel.

TABLE IV.

Results of an experiment to show that osmosis may occur *from* a fluid having a higher final osmotic pressure *towards* a fluid having a lower final osmotic pressure. For description of experiment see text.

Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours
2 h. 55′ p.m.	268		
3 h. 0′	266½	1½	
5	265	1½	
10	263½	1½	
15	262	1½	
20	260	2	
25	257½	2½	1¾
30	255½	2½	
35	253½	2½	
40	252	1½	
45	250	2	
50	248	2	
55	246½	1½	1⅞
3 h. 55′	246½		
4 h. 0′	245	1½	
5	243	2	
10	241	2	
15	239	2	
20	237	2	
25	235	2	1¾
30	233	2	
35	231	2	
40	229	2	
45	227	2	
50	224½	2½	
55	221½	3	2½
4 h. 55′	221½		
5 h. 0′	219	2½	
5	216½	2½	
10	214	2½	
15	211	3	
20	208	3	
25	205	3	2¾
30	202	3	
35	199	3	
40	196½	2½	
45	193½	2¾	
50	191½	2	
55	190	1½	2½

Freezing point of Reservoir fluid at end = −0·201° C.
„ „ Funnel „ = −0·319° C.

This is seen to have actually occurred. It will be noticed that the freezing point of the funnel fluid (sodium chloride solution) was at the end of the experiment lower than that of the reservoir fluid (glucose

solution): in other words, the final osmotic pressure of the fluid *from* which the water flowed by osmosis was throughout the experiment greater than that of the fluid *to* which the water passed.

In order to test the condition of the membrane after a long series of experiments had been made with it, it was necessary after careful washing to again fill it with one of the solutions which had earliest been investigated and determine whether an approximately similar result was now obtained. The word "approximately" is used advisedly, for it was the results derived in this way which led to the special investigation of small quantities of albumen. It is obviously impossible to remove all traces of proteid material from the inside of the instrument and particularly from the surfaces of the membrane without taking the whole instrument to pieces, and this from the nature of the result aimed at is impossible. Repeated washing with distilled water is the sole means at our disposal. Below is given in full the result obtained in the control experiment made with the membrane which had been used in obtaining the results set out in Tables I, II and III. The substance used was sodium chloride and the osmometer was washed as thoroughly as possible with distilled water.

It is seen that the initial rate of osmosis is smaller by a considerable amount than when no albumen had been used in the osmometer, while it is slightly greater (as shown by the total amount of flow in 3 hours) than when a ·1 p.c. albuminous solution is used. It is obvious however that the membrane had not been injured during the experiment, since the result obtained compares fairly well—allowing for the reduction in rate—with the result obtained at the commencement of the whole experiment. In any case no actual holes had made their appearance in the membrane, since these would have caused an increase in the rate of osmosis (for a time) and not a diminution.

Since, as has already been said, one would *a priori* expect that the initial rates of osmosis should show some constant ratio to the final osmotic pressures, and this is seen not to be the case, it became necessary to consider the question of dialysis, since it was possible that a far greater amount of dialysis in the case of salt and of urea might lead to an apparent diminution in the rates of osmotic flow of these two substances as compared with that of glucose. At the end of each experiment therefore reservoir and funnel fluids were emptied into stoppered bottles, the freezing points taken and the quantity of substance on each side of the membrane quantitively estimated. The results are to be found at the bottom of each column on the tables. Quantitive estimation was

TABLE V.

Results obtained upon initial rates of osmosis of Sodium Chloride with water as solvent and as fluid on the other side of the membrane, after the initial rates of osmosis of various albuminous solutions had been investigated. To be compared with columns 1 and 2 of Table II.

Time	Position of fluid in stem (millimetres)	Distance in mm. travelled by fluid in preceding 5 min.	Average rate per 5 min. during half-hours
2 h. 5′	0		
10	3½	3½	
15	6¾	3¼	
20	10½	3¾	
25	13	2½	
30	16	3	
35	20½	3½	3 5/12
40	23¾	3¼	
45	27	3¼	
50	30¼	3¼	
55	32½	2¼	
3 h. 0′	36½	3¾	
5	38¾	2¼	3 1/24
3 h. 5′	38¾		
10	41½	2¾	
15	44	2½	
20	46¾	2¾	
25	48	1¼	
30	50¾	2¾	
35	53	2¼	2⅝
40	55¼	2¼	
45	57½	2¼	
50	59¾	2¼	
55	63	3¼	
4 h. 0′	65½	2½	
5	67½	2	2 1/17
4 h. 5′	67½		
10	70	2½	
15	72½	2½	
20	75	2½	
25	77	2	
30	79½	2½	
35	81¾	2¼	2⅛
40	84½	2¾	
45	87	2½	
50	89	2	
55	91	2	
5 h. 0′	93	2	
5	95½	2½	2 7/24

Freezing point of Funnel fluid at beginning = −0·351° C.
" " " " end = −0·281° C.
Freezing point of Reservoir fluid at beginning = 0° C.
" " " " end = −0·009° C.
Total amount of NaCl in apparatus at beginning = ·068 gm.
Amount of NaCl found at end (Funnel) = ·0547 gm.
" " " (Reservoir) = ·013 gm.
Percentage composition of Funnel fluid at end = ·468 gm.
" " Reservoir " = ·013 gm.
Partial osmotic tension of NaCl at end is that amount which leads to freezing point −0·272° C.

not made in the case of the solutions in serum because of the difficulty introduced by the complex nature of the fluid; the freezing point was alone determined. If we consider the freezing points first we shall have an idea of the relative final osmotic pressures of the system. By subtracting the amount of lowering of freezing point observed in the case of the reservoir fluid from the amount observed in the case of the funnel fluid the freezing point of the amount of substance available at the end of an experiment for osmotic purposes, *i.e.* the partial osmotic tension, will be obtained. These are given below.

	Watery.	·1 p.c. albuminous.	Serum.
Sodium chloride	− 0·265° C.	− 0·263° C.	− 0·276° C.
Glucose	− 0·175° C.	− 0·178° C.	− 0·181° C.
Urea	− 0·15° C.	− 0·153° C.	− 0·154° C.

It is obvious therefore that the final osmotic pressure of the NaCl solution is still considerably greater than that of either the glucose or urea. With regard to the urea solution this indeed is less than that of the glucose solution, but the ratio between the freezing points is only about 5 : 6, whereas the ratio between the initial rates of osmosis of the two substances during the last half-hour of the experiment is about 1 : 5. It follows therefore that the results obtained do not depend upon the amount of the substance which has dialysed, unless we assume that the rates of flow observed are the balance between osmosis in the one direction and a flow of water brought about by dialysis in the other direction. One would then have to regard a dialysing molecule as carrying in its train a number of water molecules, that number varying with the individual crystalloid, but whether this be the case or no it is impossible at present to say, and for our purpose it is immaterial.

If we consider the actual amounts of the substances which dialysed in watery and in albuminous solutions we shall see that dialysis as well as osmosis is impeded by the presence of albumen in the solutions, but to nothing like the same extent. Were it not for the almost complete regularity of the observation that the freezing points of the faintly albuminous solutions and solutions in serum at the end of an experiment are lower than they are when watery solutions are used, the observed figures might be regarded as experimental error, but when these are corroborated by quantitive estimation of the funnel and reservoir fluids and it is found that by all three methods the effect of ·1 p.c. albumen shows itself by

impeded dialysis it must be concluded that in the case of glucose and urea at all events the presence of albumen hinders osmosis, though not to any great extent. As far as sodium chloride is concerned there is more doubt, and upon the question of dialysis in albuminous solutions it is more difficult to speak because of the presence of organic material along with the sodium chloride which renders impossible a satisfactory quantitive estimation by means of silver nitrate.

SECTION V. *Conclusions.*

The chief conclusions to be drawn from this investigation therefore are as follows:—

(1) The ratio between the initial rates of osmosis of glucose, sodium chloride, and urea in equimolecular solutions is not the same as the ratio between their final osmotic pressures or the ratio between the differences of their freezing points and that of water. The practical bearing of this conclusion is that it is impossible to state from a determination of their freezing points that one solution is hypertonic, isotonic or hypotonic as regards another solution of a different composition at pressures within the limits possible in the animal body. This can only be determined so far as is at present known by actual experiment in each individual case.

(2) The ratio between the initial rates of osmosis of glucose, sodium chloride and urea in equimolecular solutions, when the membrane is copper ferrocyanide, is not the same as it is when the membrane is prepared peritoneal membrane. It follows that general deductions as to osmosis true in the case of one membrane are not necessarily true in the case of another membrane. The importance of the membrane in determining osmosis has already been insisted on by Graham and Lothar Meyer, but has not as yet received sufficient attention.

(3) In the case of prepared peritoneal membrane the initial rate of osmosis of glucose, of sodium chloride and of urea is diminished by the presence of albumen in the solution even if that albumen be only present in very small quantities.

(4) In the case of prepared peritoneal membrane, in watery, in faintly albuminous and in highly albuminous equimolecular solutions, the initial rate of osmosis of glucose is greater than that of sodium chloride, and the initial rate of osmosis of sodium chloride greater than that of urea.

It is with much pleasure that I acknowledge the unwearying help and kind criticism given me throughout the entire investigation by Mr J. W. Capstick, Fellow of and Lecturer on Physics at Trinity College, Cambridge; without that help and criticism I should not have ventured to carry out a research upon what is, after all, a physical problem.

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ON THE RELATIONSHIP OF THE LIVER TO FATS.

By D. NOËL PATON, M.D., B.Sc., F.R.C.P. (*Edin.*), *Superintendent of the Research Laboratory of the Royal College of Physicians of Edinburgh.*

SINCE Bernard in 1855 showed that the liver, in its sugar-producing function is connected with the general metabolism of the body, the view of the older physiologists that the organ is simply a bile-forming gland has steadily lost ground. Subsequent researches have confirmed and extended the conclusion of Bernard, and at present the liver must be regarded rather as the great regulator of the supply of carbohydrate and of proteid material to the body generally than as a digestive gland.

The liver is on the direct channel of absorption of carbohydrates and proteids, but it is not on the route of the absorption of fats. These, as shown by Munk[1], are all absorbed by the lymphatics and reach the liver only through the hepatic artery.

Has the liver then any special connection with the metabolism of fats?

Such a connection is suggested by the enormous accumulation of fat in the liver of such animals as the cod at certain seasons, and of cats and other animals in certain conditions not pathological. But so far no systematic investigation of the question has been made.

Nasse[2] in a lecture to the Rostock Naturalists' Society suggests the probable connection of the liver with fat metabolism. He points out that Hofmann found an excess of free fatty acids in the liver (with this assertion I shall have to deal more fully again) and compares its condition in this respect to the condition of sprouting seeds where the same high proportion of free fatty acids exists. For this metabolism there must be a transport of fat to the liver from the body, but Nasse is constrained to admit "Such a transport of fat to the liver, and indeed an accumulation of fat in the liver in such cases (infiltration) has as yet

[1] *Virchow's Arch.* CXXIII. 230, 484. 1891.
[2] *Biol. Centralb.* VI. 235. 1886—7.

of course only once been proved by figures, namely in phosphorus poisoning[1]."

C. Voit[2], having pointed out the clear evidence there exists of the formation of fat from carbohydrates, says—"The question of whether this formation of fat from carbohydrates occurs in all organs or in a special organ, for example the liver, must remain for further research to discover."

Langley[3] gives a series of observations on the amount and distribution of fat in the liver of frogs under different conditions, which strongly supports the view that fat is metabolised in the liver whenever the general metabolism is active. Thus, fat accumulated during the cold months disappears during the summer. Winter frogs exposed to a high temperature show a decrease of fat in the liver, the individual globules becoming smaller and disappearing. Immediately after the taking of food the hepatic fat decreases—to be later increased in amount, and later still to again fall to the normal. His observations were entirely histological.

Such speculations and observations, suggestive as they may be, are of little value without a more extended experimental basis.

In the standard text-books the statements in regard to the liver fats are few and unimportant, and are based upon insufficient and inaccurate observations.

Many papers touching on the liver fats are to be found scattered through various journals; but few of them are of any value, for, in nearly all, it is assumed that the ether extract of the liver is a measure of the liver fats.

The present work is a contribution towards the systematic study of the relationship of the liver to the metabolism of fats. The methods of analyses are exceedingly tedious; and hence, though the work has extended over nearly two years, the number of observations is comparatively limited. I have, however, endeavoured, by carefully planning the experiments, and by taking every precaution, to ensure accuracy in results and to make each observation reliable.

Before approaching the subject a preliminary series of observations on the methods to be employed and the character of the fatty compounds in the liver was necessary, and the paper is thus divided into two parts:—

[1] Leo. *Zeitschr. f. phys. Chem.* IX. 469. 1885.
[2] *Biol. Centralb.* VI. p. 243. 1886—87.
[3] *Proc. Royal Soc.* XXXIX. 235. 1885.

I. Preliminary.

1. Method of extracting Fats and of estimating Fatty Acids.
2. Is the ether extract uniformly distributed throughout the Liver?
3. In animals in the same condition is the ether extract uniform in amount?
4. The composition of the ether extract.
   - A. Fatty Acids. 1. Methods. 2. Amount. 3. Relative Proportions. 4. Lower Fatty Acids.
   - B. Compounds of Fatty Acids. 1. Lecithin. 2. Free Fatty Acids. 3. Cholesterin. 4. Fats.
   - C. Other Substances.

II. Relationship of Liver to Fats.

1. Influence of Starvation.
2. Influence of Food.
   - A. Fats.
   - B. Carboyhdrates.
   - C. Proteids.

PART I.

I. Method of extracting "Fats" and of estimating Fatty Acids.

The animal, if small (*e.g.* rabbit, rat, pigeon, kitten and pup), was killed either by decapitation or by a blow behind the head. In the latter case the carotids were cut and the animal was thoroughly bled. In the case of cats the animal was rapidly chloroformed and the carotids cut. The liver was quickly excised. It was usually almost free from blood. The gall bladder was removed and the liver at once weighed. Either the whole organ or a part of suitable size carefully weighed was cut into small pieces and placed in methylated spirit for a week or more. The spirit was then poured off into an evaporating basin and the liver was pounded in a mortar and thrown again into the spirit with which it was mixed. The whole was then dried on a water bath, the substance in the evaporating basin not rising above 80° C. During this process it assumed a much darker colour and became of a fine crumbly porous consistence. For the extraction of fats, Soxhlet's method was employed. The dry residue was put in a folded filter paper into a Soxhlet s fat extractor—the basin being well washed with

ether and this ether also placed in the extractor. The extraction was carried out for at least twelve hours, the ether passing over the fat at least 40 times.

The completeness of the extraction of the compounds of fatty acids soluble in ether depends largely upon the fineness of the subdivision of the dried tissue. The liver treated in the method above described forms a fine porous crumbly material which allows of a very complete action of the ether upon it.

Since this research was completed Dormeyer[1] has contended that a very large residue soluble in ether remains unextracted from the tissues even after several days. That this is the case with certain organs there can be no doubt. But in the liver prepared and extracted as above described, the residue is very small indeed, as is shown by Exp. 78, where in 25·6 grm. of the liver of a kitten fed on cream 10·294 grm. of ether extract was obtained in the usual manner. The residue after drying was pounded with alcohol, dried and re-extracted for 12 hours. It yielded only 0·045 grm.—0·4 % of the ether extract.

Fatty Acid Compounds insoluble in Ether. But fatty acid may also exist in combinations insoluble in ether. Of these the most important are the *Soaps*.

Munk and Rosenstein[2], in their investigation on the fats of the lymph, state that soaps occur to the amount of about 5 % of the fat, *i.e.* of the ether extract. Their method is to pound the extracted residue in a mortar with alcohol and hydrochloric acid and to re-extract in a Soxhlet's apparatus. The material so extracted is considered by them the fatty acid of the soaps.

After a very careful trial I am by no means satisfied with this method. The drying with hydrochloric acid brings about a decomposition in the liver substance which leads to the formation of a tar-like substance extracted by ether but soluble in water. By Munk's method this would be reckoned as fatty acid derived from soaps. Again I find that heating fatty acids with hydrochloric acid decomposes them, and hence the ether extract cannot yield all the fatty acids set free.

The following results show that a considerable quantity of material may be extracted from the residue of the liver by Munk's method.

Exp. 62. Liver of cat weighing 64 grms. Ether extract 3·047 grms. Ether extract of residue after further treatment with hydrochloric acid 0·359 grms. = 11·8 % of the ether extract.

[1] *Pflüger's Arch.* LXI. 341. 1895.

[2] *Virchow's Arch.* CXXIII. 230—484. 1891.

Exp. 60. Liver of large fat rabbit weighing 68 grms. Ether extract 4·452 grms. Fatty acids in this 3·09 grms. Ether extract of residue, after further treatment with hydrochloric acid, 0·37 grms. = 8·3 % of ether extract.

To conclude that these are derived from soaps is hardly justifiable, for the possibility of their being produced by the decomposition of such a material as Liebermann's *lecithin albumin*[1] must be borne in mind.

The following observation, while showing that a certain quantity of the fatty acids so extracted are derived from the decomposition of soaps, would seem to indicate that some at least is formed from other sources.

The residue after extraction with ether of experiment 63 *B* was powdered and divided into two parts. *A* weighed 6·712 grms. *B* weighed 4·433 grms.

A was repeatedly extracted with cold and then with warm methylated spirit to remove soaps as thoroughly as possible. It was then rubbed up with methylated spirit and hydrochloric acid and dried.

B was at once rubbed up with methylated spirit and hydrochloric acid and dried.

Both were extracted by Soxhlet's method. Extract from

A weighed 0·115 grms. = 1·71 % of solids.

B weighed 0·195 grms. = 4·4 % of solids.

But in this liver the ether extract was 33·3 % of the solids, so the residue extracted amounted to—

A = 5·13 % of the ether extract.

B = 13·2 % „ „

The difference—8·07 % of the ether extract—must have been comprised of fatty acids combined as soaps; the remaining 5·13 % may have been produced by the decomposition of such compounds as lecithin albumins or nucleins.

The possible formation of fatty acids from nucleins is especially interesting in connection with the demonstration by Hammersten[2] and Kossel[3] of the formation of carbohydrates from these bodies; and would seem to indicate that their metabolism plays a much more important part than is usually ascribed to them in the production of the normal constituents of the body.

After extraction with ether, the action of a mineral acid liberates a further amount of material soluble in ether (fatty acids) which is in part derived from soaps and in part from such compounds as nucleins or lecithin albumins.

[1] *Pflüger's Arch.* L. and LIV. 1891 and 1893.

[2] *Zeitsch. f. phys. Chem.* XIX. 19. 1894. [3] *Arch. f. (Anat. u.) Phys.* 1893. 157.

The present research is concerned with the Fatty Acids extracted without the addition of an acid.

II. Uniform distribution of Ether Extract.

The first question to be decided is :—

Is the distribution of ether extract equal throughout every part of the liver?

Weighed portions of the liver from opposite lobes were extracted. The following table gives the percentage amount of ether extract in each part, and shows that throughout the various parts of the liver there is a uniform distribution of the ether extract, so that any part of the organ may be taken for analysis.

Table I.

Uniformity in the distribution of Ether Extract throughout the Liver.

Exp.	Animal	Ether extract % in left Lobe	Ether extract % in right Lobe
II	Sheep	9·78	9·64
III	Pig	4·74	4·58
IV	Ling	66·95	67·55
VI	Cat	5·12	5·07
XI	Cat	15·02	15·54

III. Uniformity in amount of Ether Extract in animals in the same condition.

It next became necessary to ascertain whether the ether extract is the same in the livers of animals in the same condition. In the case of glycogen it has been shown to be difficult to secure that the percentage amount of the substance in any two livers shall be anything like equal, even in animals kept on the same diet. In the following experiments the animals were kept on the same diet and under the same conditions.

TABLE II.

Uniformity in amount of Ether Extract in animals in the same condition.

Exp.	Animal	Per cent. of ether extract in Liver	Per cent. of ether extract in solids of Liver	Remarks
XII	Kittens	3·86	13·83	Suckled—two or three weeks old
		3·85	14·07	
XXV	„	5·75	24·7	Suckled—a few days old
		6·01	24·7	
		5·05	20·9	
XV	Pigeons	3·0	14·50	Starved 4 days
		5·18	14·87	
		5·44	17·58	Fed on peas for 4 days
		5·44	17·19	
		5·46	18·4	Fed on rice and suet for 4 days
		5·96	19·5	
XXXIII	Pups	6·20	24·9	Starved for about 24 hours
		6·33	24·2	„ „ „ „ „
		6·08	23·6	„ „ „ „ „
		6·14	24·0	„ „ „ „ „
XXIV	Rabbits	15·20	42·2	33 days old. Suckled for 19 days,
		18·36	47·6	then fed for 14 days on green
		16·50	45·8	food
XXVII	„	3·94	13·7	Fed on oats, water, and starch
		4·56	16·0	
		4·19	14·5	Fed on oats and water
		4·31	15·2	
XXIX	„	5·43	19·08	Fed on green food with 10 grms.
		5·46	19·30	of starch daily
		4·87	16·9	Fed on green food with 10 grms.
		4·72	16·6	dried white of egg daily
XXX	„	4·8	16·6	Bran and water + 10 grms. starch
		4·8	16·9	„ „ + 10 grms. dried white of egg
XXXVI	„	4·51	17·2	Fed on green food, oats and bran
		4·31	16·7	„ „ „ „ „
		5·02	19·2	Fed on milk
		4·90	18·5	„ „

These experiments show that in animals in the same condition the percentage amount of substances soluble in ether is fairly uniform.

IV. Composition of the Ether Extract of the Liver.

The essential part of a fat, not only from the chemical but also from the physiological standpoint, is the fatty acid.

Munk has demonstrated that a given weight of fatty acid is of equal value, as a source of energy in the body, with the corresponding amount of its glycerin fat. It is, therefore, *the amount of fatty acid which must be taken as a measure of the amount of fat in the organ.*

It was thus necessary to determine what proportion of the ether extract is formed of fatty acids.

A. *Fatty Acids in Ether Extract of Liver.*

(1) *Methods.* The ether extract in a wide mouthed flask was evaporated to dryness on a water bath and in a dry air chamber at 100° C. and weighed. Three modes of estimating the fatty acids were tried—that recommended by Lebedeff[1], that of Hoppe-Seyler[2], and that described by Kossel and Obermüller[3]. The two last were tested against one another on the subcutaneous fat of a cat with the following results.

Comparison of the method of Hoppe-Seyler with that of Kossel and Obermüller for the estimation of fatty acids.

Subcutaneous "Fat" of Cat extracted with Ether.

Method	Amount of ether extract in grms.	Fatty acids in grms.	% of fatty acids in ether extract
Hoppe-Seyler	5·71	5·206	91·0
Kossel & Obermüller	3·844	3·510	91·5

In the "Fats" of a lipoma Ruppel[4] found 89·5 per cent. of fatty acids and Schulz[5] 94·0 per cent.

Lebedeff's method was tested against these on the liver fats.

The method found most convenient was a modification of that of Kossel and Obermüller. The dried and weighed ether extract was dissolved in ether in the flask, and an excess of metallic sodium in small fragments was added, and the whole shaken from time to time. Saponification proceeded rapidly, and the soaps separated as a fine granular precipitate. The closed flask was left standing for 12 hours, and the contents were then thrown on a filter paper and well washed with ether. The precipitate was then removed from the

[1] *Zeitsch. f. phys. Chem.* VI. 189. 1882.
[2] *Chemische Analyse*, 2nd Edit.
[3] *Zeitsch. f. phys. Chem.* XIV. 599. 1890.
[4] *Zeitsch. f. Biol.* XXXI. 101. 1895.
[5] *Pflüger's Arch.* LV. 231. 1894.

paper and dissolved in distilled water in a beaker, the flask being also washed out with water and the washings added to the contents of the beaker. After complete solution of the soaps, the fluid was freely acidified with hydrochloric acid and the freed fatty acids extracted by repeatedly shaking with ether in a separation funnel. The filtrate of ether from the saponified fats was found to contain a varying proportion of soaps. It was, therefore, shaken with water to dissolve the soaps, and the watery solution was removed by means of a separation funnel from the ether which contained the cholesterin. The soaps were decomposed with hydrochloric acid and added to the separation funnel, in which the extraction of the fatty acids from the main portion of the soaps, was carried out. The ether was distilled off and the fatty acids dried at 100° and weighed.

The advantage of this method is that the great bulk of the soaps is at once freed from cholesterin, and does not require the repeated washing with ether, which is not only troublesome and expensive, but which is also apt to remove a certain amount of the soaps. The ether filtrate contains all the cholesterin.

On p. 187 it is shown that by simple washing with water it is impossible to remove all the soaps from the cholesterin. For this reason in some of the later experiments, after it had been demonstrated that the amount of cholesterin in the liver is smaller than is usually stated, the cholesterin was not separated from the fatty acids but was weighed along with them.

To make sure that the fatty acids are entirely extracted by the washings with ether, I have repeatedly tested by again extracting with ether. In each case on evaporating, a mere smear on the inside of the glass, entirely unweighable, was obtained.

(2) *Amounts of Fatty Acids in Ether Extract of Liver.*

The following table shows the average percentage of fatty acids in the ether extract of the liver of various animals. The Appendix gives the experiments upon which this table is based.

TABLE III.

Average amount of Fatty Acids in Ether Extract of the Liver.

Animal	No. of observations	% of fatty acids	Animal	No. of observations	% of fatty acids
Kitten	19	55·5	Sheep	1	66·2
Cat	3	53·1	Pigeon	3	61·3
Rabbit	31	57·8	Skate	1	65·9
Rat	6	54·6	Ling	1	69·1

These results clearly show that the ether extract of the liver gives a most imperfect indication of the amount of the essential constituents of the fats—the fatty acids.

While the ether extract of the subcutaneous and other adipose tissues is almost entirely composed of neutral fats containing 90 to 94 per cent. of fatty acids, the ether extract of the liver contains from 40 to 90 per cent., indicating that other compounds besides the normal fats must be present. What the nature of these compounds is will be considered later.

(3) *Relative Proportions of the Fatty Acids to one another.*

The melting point of a fat depends upon the amount of oleic acid contained. The greater the amount of oleic acid with the low melting point of about 6° C., and the smaller the amounts of stearic and palmitic acids with their melting points of 69° C. and 62° C. the lower will be the melting point of the fat.

The work of previous observers has tended to the conclusion that, even in the same animal, the proportion of oleic acid is not the same in the fats of all parts of the body.

Hoppe-Seyler[1] gives on the authority of Chevreul the following statement as to the melting point and point of congelation of human fat:

	Melting point	Congelation point
Fat of Panniculus adiposus	20°—22°	12°—15°
	15°—18°	6°— 7°
Fat in region of kidney	25°	17°

From Henneberg[2] he makes the following statement as to sheep's fat:

	Melting Point.
Subcutaneous Fat	27°—31°
Kidney Fat	37°—43°

So far as I am aware, the only observation on the proportion of the fatty acids in liver fat is recorded by Lebedeff[3], who states that in human fat palmitic and stearic bear to oleic acid the following proportions:

Subcutaneous Fat	1 to 5·11
Intestinal Fat	1 to 3·50
Liver Fat	1 to 1·85[4].

[1] *Physiol. Chem.* 629. [2] *Ibid.* p. 630.

[3] *Zeitsch. f. phys. Chem.* VI. 139. 1882.

[4] The results of Länger quoted in Beilstein's *Organic Chemistry*, I. 334, agree with these.

These figures would indicate the presence of a comparatively small proportion of oleic acid in the liver fats. These observations are, however, insufficient and the question required further investigation.

In a lipoma Ruppel[1] by cooling found the stearic and palmitic acids were to oleic acid in about the proportion of 1 to 3, while Schulz[2] by v. Hüble's method found the proportion about 1 to 2.

In investigating this point two methods have been employed.

I. The ether extract was made in the usual way. Saponification was carried out by Hoppe-Seyler's or by Kossel and Obermüller's method. The fatty acids were separated by Lebedeff's method[3]. The solution of soaps was acidified with acetic acid, and precipitated with acetate of lead. The precipitate was washed and dried at a low temperature over H_2SO_4. The oleate of lead was then extracted with ether, decomposed with H_2S and the oleic acid recovered by evaporating the ether. The palmitate and stearate of lead were rubbed up in a mortar with absolute alcohol and decomposed with H_2S. The alcohol was evaporated and the fatty acids extracted with ether.

The following results were obtained:

TABLE IV.

Proportion of Palmitic and Stearic Acids to Oleic Acid.

Exp.	Animal	Proportion of palmitic & stearic acids to oleic acid	Method
IV	Cat	1 : 1·3	Lebedeff's
XI	,,	,, 1·4 / 1·3	,,
IX	Child	,, 1·65	,,
XIII	Rabbit	,, 2·06	,,
VIII	Skate	,, 3·27	,,
V	Ling	,, 3·34 / 3·84	,, Decant. of oleic acid

The method is tedious and unadapted for any prolonged series of observation. It also requires a considerable amount of fatty acids.

II. The melting point of a mixture of these acids gives an index of the relative proportion of oleic and of palmitic and stearic acids.

[1] *Zeitsch. f. Biol.* XXXI. 101. 1895.
[2] *Pflüger's Arch.* LV. 231. 1894. [3] *Loc. cit.*

This is pointed out in Allen's *Organic Commercial Analysis*, Vol. II. p. 224, where the following table based on the observation of Delicare and Jean with mixtures of pure commercial stearic and oleic acids is given.

Melting point	% of stearic acid	Melting point	% of stearic acid
40° C.	35·15	44° C.	47·5
41 „	38	45 „	51·3
42 „	39·9	46 „	53·2
43 „	43·7	47 „	57·9

The determination of the melting point of these acids when in small amount may be readily effected by placing them in capillary glass tubes which may be dipped into vessels containing water at different temperatures. A series of capillary tubes of the same calibre are prepared, the acids to be investigated are melted over the water bath and drawn into the tubes. These are then sealed by placing the end in a gas flame.

Observations were made with commercial stearic and oleic acids. Commercial stearic acid is really a mixture of stearic and palmitic acids and has a melting point of 55° C. According to Heinz's observations this corresponds to a proportion of 30 parts of stearic acid to 70 of palmitic acid. The oleic acid is nearly pure and melts at 7° C. Mixtures of these were made in the proportion given in the first column below and the melting points given in the second column were determined.

% of stearic and palmitic acids in mixture	Melting point
50	46° C.
33	37 „
25	34 „
20	28 „
16·6	24 „

In its upper figures this table corresponds with that of Delicare and Jean.

The following results obtained by this method confirm those yielded by Lebedeff's procedure.

TABLE V.

Fatty acids of	Melting point	% of palmitic and stearic acid
Rabbit		
Body fat	24—25° C.	16·6
Liver fat	36—37 „	30
Kitten (young)		
Body fat	26—27° C.	20
Liver fat	41—45 „	36
Porpoise		
Body fat	11° C.	?
Liver fat	39 „	34

The fats of the liver are thus distinguished by containing a much smaller proportion of oleic acid than the fats of the other parts of the body.

This may be due to one of two different causes. Either the oleic acid being less saturated is more rapidly metabolised in the liver, or the proportion of the acids may depend upon the temperature of the tissue, being such as to insure that at that temperature the fat shall be fluid. This latter view seems to be supported by the varying melting point of the subcutaneous fats in different animals. In the porpoise living in a medium of low temperature and with a low peripheral temperature the subcutaneous fats are specially rich in oleic acid.

(4) *Lower Fatty Acids.*

The occurrence of lower and of volatile fatty acids, which has been demonstrated by Schulz in the subcutaneous fat of a lipoma, was not investigated. They seem to occur in such small amounts as to be of practically no physiological significance.

B. *Compounds of the Fatty Acids.*

In ordinary adipose tissue the fatty acids are almost entirely combined with glycerol as neutral fats. But it has been already shown that in the liver other compounds occur.

I. *Lecithin.*

Of these undoubtedly the most important is Lecithin—a compound in which phosphoric acid linked to cholin replaces one of the molecules of the fatty acid radicle in a neutral fat.

This compound yields about 54% of fatty acids as stearic acid, 15% of cholin, and 12% of phosphoric acid.

The work of previous observers indicates that lecithins are important constituents of the liver.

Lebedeff[1] says: "I have succeeded in ascertaining that, on a diet rich in nitrogen and poor in fat, no fat is present in the liver. I thus found in geese which were fed for a month and a half on peas, only a small collection of fat in the omentum and intestine while the feebly developed liver contained only lecithin and no fat." The method of estimating lecithin is not given.

Stolnikow[2] describes a series of observations, without, however, giving any description of his methods of analysis, which led him to the conclusion that the proportion of fatty acids combined as lecithin varies much in different conditions of the liver in frogs and is much increased in phosphorus poisoning. He gives a table of the proportion of lecithin in the fats with various diets in the poisoned and unpoisoned condition.

In 100 grms. of "Fat" he finds of lecithin:

Condition of frog	Unpoisoned	Poisoned with Phosphorus
No food	7 %	43 %
Sugar	0	50
Peptone	43	59

Without any description of the methods employed such results cannot be accepted as conclusive. From the histological changes in the liver cells he develops the theory that the phosphorus of the nuclei is carried out into the cells and there combined with fatty acids to form lecithin. The appearances he figures are certainly curious and interesting, but his work requires repetition.

A much more important communication was made by Heffter[3] in 1891. By carefully drying the liver at a low temperature he prevented any decomposition of lecithin and found in a series of eleven analyses of the liver of rabbits an average of 7·89 % of lecithin in the ether extract, and in the case of three analyses of the liver of the dog 69·6 %. Calculated in per cent. of liver substance his analyses gave a mean of 2·09 %, the greatest variations being from 1·39 to 3·07 %; or, reckoned as per cent. of the dry solids of the liver, 9·6. According to his observations in phosphorus poisoning, while the fats of the liver are increased, the lecithin is diminished.

[1] *Zeitsch. f. phys. Chem.* VI. 139. 1882.
[2] *Arch. f. (Anat. u.) Phys.* Supplem. Heft. 1887. p. 1.
[3] *Arch. f. exp. Pathologie*, XXVIII. 97. 1891.

He concludes:

"1. The amount of lecithin has a direct relationship to the amount of the liver tissue. In rabbits at least it is not modified by changes in diet. In hunger a diminution occurs.

"2. In phosphorus poisoning a distinct reduction in the amount of lecithin occurs—on an average nearly 50 %—which is more marked the greater the accumulation of fat in the liver."

In his table, p. 102, he gives side by side the amount of ether extract, of lecithin and of "fat and cholesterin." But as these last two columns, extending to the fourth point in decimals of a gramme, added together correspond exactly to the first, it is obvious that his fats and cholesterin have been arrived at by subtracting the lecithin from the ether extract on the unwarrantable assumption that these are the only constituents of the extract. My own analyses show that a very varying quantity—an average of 12 %—is not composed of these substances.

As pointed out by Heffter the great difficulty is the ease with which lecithin is broken up. An acid reaction or a temperature over 50° C. is supposed to bring about this splitting[1].

It may be urged that, if this is so, the method of drying adopted in my observations is unsatisfactory in as much as it is calculated to break up the lecithin before extraction with ether, and thus to diminish the amount of ether extract and to increase the proportion of fatty acids in it. To test this the following observation was made.

The liver of a rabbit was divided into two parts, *A* and *B*.

A weighed 38 grms. *B* weighed 29·5 grms.

A was cut into several pieces and placed in absolute alcohol. *B* cut up in the same way, was placed in methylated spirits.

Both were kept in these solutions for a week and then rubbed up in a mortar. *A* was then dried during 48 hours in an incubator at 38° C. *B* was dried during 8 hours on a water bath.

The dry residue of each was extracted by Soxhlet's method.

TABLE VI.

	Ether extract		Fatty acids			Lecithin		
Exp.	In grms.	%	In grms.	% of liver	% of ether extr.	In grms.	% of liver	% of ether extr.
A	1·761	4·68	1·073	2·82	60·9	0·989	2·6	56·1
B	1·377	4·67	Lost	—	—	0·807	2·7	58·4

[1] *Loc. cit.*

Such an observation shows that my method of drying and extracting the liver does not decompose the lecithin.

1. Methods. In the estimation of the lecithin two different methods were used.

A. A weighed portion of the ether extract of the liver was directly burned with potassic hydrate and nitre, these reagents having previously been ascertained to be free of phosphorus. The fused white mass was dissolved in water, neutralized with acetic acid, and filtered through paper previously extracted with dilute hydrochloric acid to remove phosphates. The filter paper was well washed with water. The filtrate was rendered alkaline with ammonia, and the phosphoric acid precipitated with magnesia mixture, allowed to stand for 24 hours, and the precipitate collected on a small ash free filter paper, washed with water containing ammonia, and ignited. The weight of the ash of phosphate of magnesia multiplied by 7·27 gave the weight of lecithin.

B. The ether extract was saponified, acidified, and the fatty acids extracted with ether. The watery residue was then evaporated to dryness and treated as above.

2. Amount. The following table gives the amount of lecithin in the liver of various animals in different conditions.

TABLE VII.

Lecithin in Liver.

Exp.	% of liver	% of solids of liver	% of ether ext. of liver	Animal	Remarks
65	2·60	11·4	56·1	Rabbit	Fed on bran and oats
	2·70	11·9	58·4	,,	,, ,,
73	1·90	—	36·4	Cat	
74	2·05	—	51·3	Rabbit	Fed on bran and green food
75 *A*	2·57	9·1	50·0	Kitten	Fed on bread and milk
B	3·04	10·3	60·0	,,	48 hrs hunger
C	3·31	11·7	70·0	,,	59 hrs hunger
D	1·72	5·2	11·0	,,	24 hrs after cream
78	1·42	9·8	3·53	,,	Fed for week on cream
59	2·24	8·9	34·2	Rabbit	Fed on turnips—very fat

These results correspond closely with those of Heffter, and show that lecithin is a constant constituent of the liver and that its amount is fairly constant averaging 2·35 % of the liver and 9·7 % of the solids of the organ. On the other hand, the

proportion of lecithin in the ether extract varies greatly, being largest during starvation and smallest on a fatty diet.

In normal states of nutrition the amount of lecithin bears a fairly fixed proportion to the total amount of fatty acids, *i.e.* a fairly fixed proportion, about one-half, of these fatty acids is combined in lecithin.

TABLE VIII.

Exp.	Lecithin	Fatty acids	$\frac{\text{Fatty acid}}{\text{Lecithin}}$	Remarks
65	2·60	2·82	1·08	Rabbit
74	2·05	2·45	1·14	„
59	3·29	4·4	1·30	„
73	1·9	3·2	1·67	Cat
75 *A*	2·57	2·75	1·06	Kitten
B	3·04	2·9	0·96	„
C	3·31	3·63	1·09	„
D	1·72	10·6	6·1	Kitten fed on fat
78	1·42	36·8	28·3	„ „

As shown by 75 *D* and 78, when fat rapidly accumulates in the liver the proportion of lecithin sinks.

These results are opposed to the view expressed by Lebedeff, that on a proteid diet the fatty acids are entirely contained in lecithin compounds. Nor do they support the opinion of Heffter that in starvation the lecithin diminishes. His experiments certainly do not seem to justify this conclusion.

3. Mode of Occurrence in Liver. There can be no doubt that lecithin is present even when the liver cells under the microscope show no trace of fat globules. While lecithin is not soluble in water, it is nevertheless acted on by water much more markedly than fats, and undoubtedly a large amount of the lecithin must be held in solution or suspended in the paraplasm or in the protoplasm of the cells.

The researches of Liebermann[1] strongly support the view of the existence of a chemical compound with the proteids or nucleins of the cell. How close this connection may be it is difficult to decide, but the fact, that from nuclein purified in the usual way both fatty acids and phosphoric acid may be obtained, by treatment with NaHO and HCl, and subsequent extraction with ether, would seem to indicate that lecithin or some lecithin-yielding compound, is intimately linked with the nuclein.

The relationship of lecithin to the more complex substance *jecorin* cannot here be considered. It is possible that the liver lecithin may

[1] *Pflüger's Arch.* LIV. 573. 1893; *Centralb. f. med. Wissensch.* 1893, No. 28.

exist in such a molecule. But such protagon substances are so readily decomposed even by a temperature of 48° C.[1] that any jecorin present must be largely broken up in the process of drying the liver.

Heffter[2] failed to find sulphur in the ether extract of eight livers. I find that sulphur is sometimes present and sometimes absent. Since the ether used in extracting was not *absolutely* water free and might therefore dissolve jecorin, it would seem that the presence or absence of sulphur depends upon whether the jecorin molecule has or has not been decomposed by the previous heating. In any case the fatty acids would appear in the ether extract and hence the presence of jecorin does not interfere with the estimation of these substances.

Two experiments are given to show the occurrence and non-occurrence of sulphur. The various reagents were first tested for sulphur with negative results. The ether extract was then ignited with potassic hydrate and nitre, dissolved in water, neutralized with acetic acid, filtered, evaporated to a small volume, rendered strongly acid with hydrochloric acid and treated while hot with a saturated solution of nitrate of barium. The precipitate was collected on a small ash-free filter-paper and ignited.

Exp. 60. Liver of rabbit weighed 60 grms.

Ether extract	2·22	grms.	treated as above
$BaSO_4$	= 0·023	„	
S	= 0·00315	„	
	= 0·141	„	% of ether extract
S in jecorin	= 2·14 %		
∴ jecorin	= 6·59 %	„	of ether extract.

Exp. 78. Liver of kitten fed on cream.

In 2 grms. of the ether extract no precipitate of the $BaSO_4$ was obtained.

4. Significance. That lecithin, or the phosphorus of lecithin, is of use in the formation of the nucleins of the body, is shown by the development of these from the yolk of the egg in birds and other oviparous animals. The value of fats in the treatment of rickets seems still further to connect them with the utilization of phosphorus.

The large and constant amount of lecithin in the liver and the power which the organ possesses to store or manufacture fatty acids seem to point to its having the power of synthesising these with phosphoric acid and cholin into the lecithin molecule as a first stage to the utilization of the former in the construction of nucleins.

[1] Neumeister, *Physiol. Chemie*, Bd. II. 68. [2] *Loc. cit.*

The evidence of such synthetic processes occurring in the liver is now quite satisfactory both as regards glycogen and urea[1].

As to the source of the phosphorus, it is highly probable that this is yielded by retrogressive metabolism of the nucleins, and that just as the liver has the power of retaining iron to be again built into the hæmoglobin molecule so it can also retain the phosphorus for the construction of lecithin.

But it is also possible that the phosphorus absorbed as salts of phosphoric acid in the food may in the liver go to the production of lecithin.

The liver would thus seem to have this important function of utilizing and economising the phosphorus of the body by combining it with fatty acids to form lecithin as a stage in its conversion into the nuclein compounds of the body.

This conception of the nature of hepatic lecithin opens out a number of distinct lines of investigation, among the most important of which is the amount of lecithin in the fœtal liver.

II. *Free Fatty Acids.*

Hofmann[2] describes a method for determining the amount of free fatty acids. Of the fats, procured by gently heating the tissue in which they were contained, a small quantity, from 2 to 20 grammes, was placed in a small thin-walled flask and weighed. They were then dissolved in ether of neutral reaction, and titrated with a freshly prepared alcoholic solution of hydrate of soda standardised against $\frac{N}{10}H_2SO_4$. He states that,

100 parts stearic acid = 14·80 H_2SO_4,
100 parts palmitic acid = 15·62 „ ,
100 parts oleic acid = 14·18 „ ,

and finds that while the fats of fatty tissue have an acidity corresponding to 0·003 in one case, and 0·062 parts in another, of H_2SO_4 per 100 parts of fat, the fats of the liver have an acidity as high as from 0·876 to 1·448 parts of H_2SO_4 per 100 parts of fats.

Nasse[3] quotes this observation of Hofmann, and considers that the high proportion of free fatty acids points to an active metabolism of fats in the organ. He compares the condition to that in young sprouting seeds, where also the free fatty acids are in excess.

[1] Pflüger. *Pflüger's Arch.* 1888.
[2] *Beiträge z. Anat. u. Phys. als Festgabe Carl Ludwig gewidmet*, 134. 1874.
[3] *Biol. Centralb.* VI. 235. 1886—87.

From the acidity of the ether extract the amount of free fatty acids cannot be deduced. Hofmann himself admits, p. 147, that other acids besides the fatty acids present in the liver fats may be overlooked by this determination of acids. Other acids such as lactic acid rapidly develop in the liver and may adhere to the fats, while the lecithin present in such abundance will likewise contribute to the acidity.

Nor will washing with water to remove the lower fatty acids and glycero-phosphoric acid formed by decomposition of lecithin yield results indicative of the amount of free fatty acids, for the lecithin, when the ether is shaken with water, swells up and forms a somewhat gelatinous mass, which remains suspended in the ether and adds to its acidity.

When the lecithin is large in amount the acidity is great, when small in amount the acidity more nearly approximates to that of the body fats. This is well shown in the two succeeding observations.

Exp. 79. Of a rabbit's liver 57 grms. were extracted as above. The ether extract amounted to 2·315 grms. or 4·06 %. Of this 1·424 grms. or 66 % was composed of lecithin. 0·57 grms. of the ether extract was dissolved in ether and washed with water.

The watery solution had the acidity of	0·00237 grms. H_2SO_4
The ether solution had the acidity of	0·00558 grms. H_2SO_4
Together	0·00795 grms. H_2SO_4,

which equals 1·37 % H_2SO_4 in the ether extract.

Exp. 78. Kitten fed on cream for ten days was killed on June 7th. The liver weighed 25·6 grms. and was extracted as above. The ether extract amounted to 10·294 grms. or 40·2 %. Of this only 3·54 % was composed of lecithin.

1·03 grms. was dissolved in ether and titrated with soda solution.

Acidity = 0·00357 grms. H_2SO_4
= 0·346 % of the ether extract.

A second 1·03 grms. was dissolved in ether and washed with water.

Acidity of watery solution = 0·0009 grms. H_2SO_4
= 0·087 % H_2SO_4,
Acidity of ether solution = 0·259 % H_2SO_4.

With such a substance as lecithin present it is practically impossible to come to any conclusion as to the amount of free fatty acids present in the ether extract.

III. *Cholesterin.*

If fatty acids are combined with cholesterin the amount so combined must be small. Cholesterin being a monatomic alcohol it can link with only one molecule of the radicle of a fatty acid. Such a compound with stearyl would have one part of cholesterin to about ·8 of stearic acid.

The amount of cholesterin in the liver has been exhaustively studied by Kausch[1]. He concludes:

The amount of cholesterin in the liver and bile reckoned in the dry substance is very constant and is in both about 2 per cent. Taking his first ten cases of normal livers the cholesterin is 7·7 % of the ether extract or "fats." His method was to saponify the ether extract, to evaporate, to add water and then to extract the cholesterin with ether. The ether was finally washed with water to free it from soaps and evaporated to dryness.

My experience is that such a process does not free the cholesterin of soaps which are partly soluble in water containing ether. The residue must be finally washed with cold alcohol to remove the last residue of soaps. If this precaution is not taken results closely corresponding to those of Kausch are obtained.

The present paper does not deal with the cholesterin of the liver but the few observations I have made indicate that the amount of cholesterin is much smaller than Kausch gives, and is independent of the amount of fats in the liver.

Exp. 42. By Kausch's method.

Ether extract % of liver	Cholesterin % of liver	% of ether extract
9·09	0·9	8·08
8·68	1·0	11·5
4·39	0·44	9·9

The effect of washing with alcohol is well shown in experiment 29, where the cholesterin as estimated from the washed ether amounted to

A. 0·198 = 18·3 % of the ether extract,

B. 0·220 = 20·3 % „ „ „

but after washing with alcohol came to only

A. 0·030 = 2·9 % of the ether extract,

B. 0·033 = 3·0 % „ „ „

With this precaution the following results were obtained.

[1] "Gehalt Leber und Galle an Cholesterin unter pathologischen Verhältnissen," Strassburg Diss. 1891.

TABLE IX.

Rabbits.

Exp.	Ether extract % of liver	Cholesterin % of liver	% of ether extract
17	6·202	0·016	0·2
17 *A*	6·95	0·05	1·35
27	3·94	0·02	0·62
	4·56	0·04	0·87
	4·31	0·05	1·30
59	6·54	0·06	0·9
Average		0·039	0·87

Cats.

11	15·02	0·05	0·71
12	3·86	0·016	0·45
	3·87	0·021	0·56
16	3·16	0·029	0·90
Average		0·029	0·65

Thus if fatty acids are combined with cholesterin they occur only to the amount of from ·031 to 0·023 per cent. of the liver substance, and they as well as the cholesterin may be neglected in estimations of the total fatty acids.

IV. *Neutral Fats.*

To determine the amount of fatty acids, combined with glycerine as neutral fats, is no easy matter in such a mixture as the ether extract of the liver. Nor has its determination any important bearing.

But by determining the amount of lecithin, the amount of fatty acids combined in this compound is readily calculated, since lecithin contains about 54 per cent. of fatty acids. The fatty acids so combined (Col. 6, Table X.) subtracted from the whole amount of fatty acids (Col. 4, Table X.) gives the amount of these substances which *may* be combined as neutral fats (Col. 7, Table X.).

Table X. shows that rather more than one-half of the fatty acids may be in the form of neutral fats.

C. *Substances other than Fatty Acid Compounds.*

The present research is not concerned with the constituents of the ether extract other than the compounds of fatty acids. Nevertheless it affords an indication of the amount of ether extract not of the nature of such compounds.

The lecithin (Col. 5, Table X.), the other fatty acids and cholesterin (Col. 7, Table X.), added together (Col. 8, Table X.) and subtracted from the whole ether extract (Col. 3, Table X.) gives an indication of the amount of other substances present.

The average amount of ether extract *not* lecithin, fatty acids or cholesterin is thus 12 %, varying from 4·2 to 22·7 % and being smallest when the proportion of fatty acids is highest, and highest when this is lowest. The chemical nature of this residue has not been investigated. In part at least it is composed of pigments.

TABLE X.

1	2	3	4	5	6	7	8	9	10
Exp.	Animal	Ether extract	Fatty acids	Lecithin	Acids as Lecithin	Acids not in Lecithin	Sum of 5 and 7	Difference of 3 and 8	% of ether ext. not lecithin, fatty acids or cholesterin
59	Rabbit	6·54	4·54	2·24	1·21	3·33	5·57	0·97	14·8
65	,,	4·68	2·82	2·60	1·40	1·42	4·02	0·66	14·1
74	,,	3·99	2·45	2·05	1·11	1·34	3·56	0·43	10·8
75	Kitten	5·09	2·75	2·57	1·39	1·36	3·93	1·16	22·7
75	,,	5·05	2·90	3·04	1·64	1·26	4·30	0·75	14·8
75	,,	4·71	2·78	3·31	1·79	0·99	4·30	0·41	8·7
75	,,	11·90	10·60	1·72	0·93	9·67	11·40	0·50	4·2
78	,,	40·2	36·8	1·42	0·77	36·03	37·45	2·75	6·8
								Average	12·1

PART II.

INFLUENCE OF VARIOUS FACTORS ON LIVER FATS.

Having considered the combinations in which the fatty acids occur in the liver and having shown that the amount of ether extract is uniform throughout the various parts of the liver, and that in animals in the same condition the ether extract and fatty acids are nearly the same, the nature of the relationship of the liver to the fats may be dealt with.

In drawing conclusions from the amount of fats and fatty acids present in the liver many difficulties are met with.

1. The percentage may vary with the amount of water present. This difficulty is easily overcome by weighing the residue, dried at 110° C., after extraction with ether, and calculating the amount of fats in terms of the dry solids.

2. The other solid constituents of the liver may vary and thus alter the percentage of fats. This is specially the case with glycogen, a substance which may vary from under 1 to over 10 per cent. of the liver substance. The influence of this factor may be excluded by estimating the glycogen.

But there is the further difficulty of ascertaining whether an increase or diminution is due to a change in the rate of accumulation—be it by production or by import from other tissues—or to a change in the rate of disappearance—be it by metabolism or by export.

Accumulation of fat. That fat may be transported to the liver and accumulated in it has been clearly shown by the experiments of Leo on phosphorus poisoning[1], by Rosenstein's observations on the fatty accumulation in phloridzin poisoning and by Exp. 47 on fatty feeding.

The very marked difference between the melting points of the liver fats and the fats of the rest of the body affords a means of determining whether in any case an import of fat has taken place. In Exp. 47, p. 198, the milk fat accumulated had a distinctly lower melting point, *i.e.* a distinctly greater amount of oleic acid, than the ordinary liver fats. The same experiment, where the melting point of the liver fat remained at 34° C. for 2 days, indicates that this method yields reliable results and that the oleic acid is not so rapidly metabolised as to cause the imported fat to at once assume the high melting point of the typical liver fats.

As to the actual production of fats in the liver, the evidence will afterwards be considered and it will be shown that it is in favour of the view that such a production occurs.

Disappearance of fat. That fat once accumulated in the liver is again got rid of is indicated by Exp. 47, p. 198. But whether the fat is simply thrown out or whether it is metabolised in the gland is not made manifest.

That such a simple conversion as takes place in the case of glycogen does not take place in the case of the fats is indicated by the fact that in the liver, kept at the body temperature after the death of the animal but during the continued life of the gland cells, no diminution in the amount of the ether extract or of the fatty acids occurs. This is shown by the three following experiments.

Exp. 21. Rabbit killed on 14th March. Liver divided into three parts.

A weighed 29·5 grms. and was placed in 0·75 % NaCl solution at 40° C. for 4 hours.

B weighed 29·5 grms. and was placed in a similar solution with chloroform for 24 hours.

C weighed 21·6 grms. and was placed in boiling water.

[1] *Loc. cit.*

All were rubbed up in a mortar, dried and extracted in Soxhlet's apparatus.

	Weight of liver	Ether extract	
		Total	% of liver
A	29·5	0·480	1·621
B	29·5	0·496	1·681
C	21·6	0·352	1·630

Exp. 48. A rabbit was killed on August 9th and the liver at once divided into two parts *A* and *B*.

A weighed 8·7 grms. and was at once placed in actively boiling water.

B weighed 17·0 grms. and was placed in 0·75 % solution of chloride of sodium and kept at 40° C. for 6 hours, and then boiled.

Each was rubbed up in a mortar and dried on the water bath and then extracted in Soxhlet's apparatus in the usual manner.

The extract was dried and weighed, then saponified and the fatty acids extracted and weighed. The cholesterin was separated by evaporating the ether filtrate of the saponified fats and washing the residue with cold alcohol.

	Weight[1] of liver	Ether extract		Fatty acids		
		Total	%	Total	% of liver	% of ether extract
A	8·7	0·342	3·93	0·212	2·43	61·9
B	17·0	0·701	4·12	0·423	2·48	62·6

Exp. 13. Rabbit killed on January 18th. Liver cut into four pieces, *A*, *B*, *C*, and *D*.

A weighed 4 grms. and was placed in boiling water at once.

B weighed 10 grms. and was placed in 0·75 % salt solution and kept at 40° C. for 4 hours.

C weighed 12·0 grms. and was placed in methylated spirits.

D weighed 12·5 grms. and was kept in 0·75 % salt solution at 40° C. for 4 hours. The salt solution was then poured off and methylated spirits added.

The glycogen was determined in *A* and *B* by Brücke's method.

After a week *C* and *D* were dried at 100° C. and extracted in Soxhlet's apparatus.

Glycogen.

	Total	% of liver
A	0·225	5·62
B	0·225	2·22

[1] Throughout this paper the weights given are in grms.

Fats.

	Ether extract		Fatty acids		
	Total	% of liver	Total	% of ether extract	% of liver
C	0·504	4·20	0·349	72·3	2·90
D	0·608	4·86	0·440	69·2	3·52

The metabolism of the fats is probably of a more complex nature than the change of glycogen. The fatty acids are largely combined in the complex phosphorus-containing lecithin, and the possibility of their being thus converted into the nuclein compounds of the living cell must be borne in mind.

The fats must be got rid of either by being first built up in the protoplasm of the liver cells and there metabolised, or by being exported from the liver as fats. The evidence on this subject will be considered in a latter part of this paper (p. 196).

The first point to be determined is the influence of abstinence from food upon the fats and fatty acids of the liver.

I. Fasting.

During starvation the liver like other organs loses weight. The glycogen rapidly disappears, but the metabolic changes are continued as is shown by the constant production of glucose, of urea, and of the various bile constituents. That the activity of metabolism is diminished is shown by the lower temperature of the blood leaving the organ, and by the diminished manufacture of the various products of the liver. This diminution in the hepatic metabolism in starvation is further demonstrated by the experiment of v. Schroeder[1] and the more recent observations of Schöndorff[2] on urea production.

Does this diminution in the metabolism lead to an increased accumulation of the liver fats, or do the needs of the organism produce a withdrawal of fats from the liver?

To elucidate these points the following experiments were performed.

Exp. 31. Three kittens of a litter of five, born on April 25th, were suckled by the mother till 12 on April 26th. *A* was killed at 3.30 ($3\frac{1}{2}$ hours after food). The stomach was full of milk. Glycogen, as indicated by the iodine staining of a scraping, was abundant in the liver.

[1] *Arch. f. exp. Path.* 15. 1881—82.

[2] *Pflüger's Arch.* 54. 1893.

B was killed at 5.40 (about 6 hours after food). The stomach contained milk, and glycogen was abundant in the liver.

C was killed at 10 a.m. on the 27th (22 hours after food). The stomach was empty, and glycogen was much less abundant in the liver.

	Weight of liver	% of solids less ether extract	Ether extract % of liver	Ether extract % of solids	Fatty acids % of ether extract	Period of fast
A	6·8	21·8	5·63	25		3½ hrs
B	7·0	21·7	5·78	26	62·9	6 „
C	5·8	21·3	7·12	33		22 „

Exp. 32. Four kittens, four days old, were taken from the mother at 8 a.m. on May 4th.

At 11 a.m. they weighed:

A	93 grms.	*C*	109 grms.
B	115 „	*D*	114 „

A and *B* were killed at once. *C* and *D* were kept without food till 1.30 on 5th (29½ hours), and then killed. *C* weighed 99 grms. *D* weighed 101 grms.

	Weight of liver	% of solids less ether extract	Ether extract % of liver	Ether extract % of solids	Fatty acids % of ether extract	Period of fast
A	3·4	19·26	4·59	23·7	67·1	0 hrs
B	3·9					
C	3·5	18·00	4·47	24·8		29½ „
D	3·7					

Exp. 42. Four young kittens of a brood procured on 20th June. They had been suckled in the morning.

A	weighed	214·5	grms.
B	„	204·5	„
C	„	214·0	„
D	„	199·0	„

A and *B* were killed at 4 p.m., about 8 hours after food. Milk was present in the stomachs of both.

C was kept without food till 5 p.m. on the 21st—33 hours—and then killed. The stomach was empty. Weight 203 grms.

D was kept without food till 2.30 p.m. on the 22nd—56½ hours—and then killed. The stomach was empty. Weight 175 grms.

The stomach and intestines were removed from each, and the rest of the body placed in methylated spirit for two weeks. The body was then minced in a mincer and extracted with hot methylated spirit. This was filtered hot. The residue was then extracted with ether and the ether added to the

alcoholic extract and evaporated to dryness on the water bath. The dry residue was then repeatedly extracted with ether and the ether distilled off, and the residue weighed. It was then saponified by Kossel and Obermüller's method and the fatty acids extracted in the usual way.

Body.

	Ether extract	Fatty acids total amount	% of weight	Period of fast
A	Lost	—	—	
B	6·855	2·537	1·27	8 hrs
C	4·835	2·475	1·21	33 „
D	4·152	2·178	1·24	56½ „

Liver.

	Weight of liver	% of solids less ether extract	Ether extract		Fatty acids		Period of fast
			% of liver	% of solids	% of liver	% of ether extract	
A	9·0	23·4	4·15	15·0	2·63	55·9	8 hrs
B	7·5	24·0	4·50	15·8			
C	6·0	20·3	[1]7·36	26·6	2·53	45·5	33 „
D	5·5	22·0	4·05	19·4			56½ „

These three experiments on kittens indicate that in the case of the usual storage of fat from ordinary food—the cat's milk—no diminution in the amount of fats occurs in the course of 56 hours.

Experiment 47 (p. 197) shows that when an excessive amount of fat has been stored in the liver, after 68 hours it has been, to a large extent, got rid of, either by metabolism within the liver or by transport from the organ. The amount of fat is then about the same as during an ordinary diet.

To investigate the influence of more prolonged fasts pigeons were used.

Exp. 50. Twelve pigeons were procured on 20th September and fed on bruised oats and water for four days.

A and *B* were killed on September 25th.
C and *D* were starved till the 26th, and then killed, 24 hours' fast.
E and *F* „ „ 27th, and then killed, 48 hours' fast.
G and *H* „ „ 29th, and then killed, 72 hours' fast.
J and *K* „ „ 30th, and then killed, 96 hours' fast.

[1] Ether extract imperfectly dried.

	Weight of liver	% of solids less ether extract	Ether extract % of liver	Ether extract % of solids	Period of fast
A	5·2	22·8	4·37	16·06	
B	6·7	21·0	4·46	17·4	
C	4·2	22·7	3·92	14·7	
D	3·5	24·1	4·31	15·1	24 hrs
E	6·2	18·0	3·25	11·1	
F	4·2	24·6	3·73	10·3	48 „
G	4·2	23·2	5·40	18·8	
H	4·5	22·4	4·46	16·6	72 „
J	2·8	24·1	4·68	16·2	
K	4·3	25·8	5·04	16·3	96 „

In this experiment the fatty acids were not estimated, but the next experiment shows that they are not diminished even after 84 hours fasting.

Exp. 35. Two pigeons kept on a similar diet of oats till June 1st. *A* had the food continued till the 5th, while *B* was starved for 84 hours. On the 5th both were killed.

		1/6	4/6	5/6
Date				
Weight:	*A*	346	378	383
	B	267	234	225

A had much fat on the body. The liver was large, pale, and stained deep brown with iodine. *B* had no visible fat on the body. The liver was small, dark brown, and hardly stained with iodine.

	Weight of liver	% of solids less ether extract	Ether extract % of liver	Ether extract % of solids	Fatty acids % of liver	Fatty acids % of ether extract	Period of fast
A	5·8	24·4	4·69	19	2·79	60	0
B	2·7	25·8	4·90	19	2·79	56	84 hrs

These results indicate that even in a fast extending over four days the normal amount of fats in the liver is not altered. But whether this indicates a non-metabolism of the fats, or whether it shows a correlated decreased accumulation and metabolism, maintaining the normal amount, is not demonstrated. It is at least certain that there is no such a disappearance of fat as of glycogen from the liver in starvation. Either by diminished metabolism, or by transport from without, or by the production within the organ, the liver maintains a store of fatty acids during inanition.

Probably one object of this is to afford the necessary material for combination with phosphorus as lecithin. During starvation the

nucleins must undergo katabolic changes and for the maintenance of the life of the cells they must be renewed. For this purpose the waste phosphorus must be retained, and to retain it a supply of fatty acids seems necessary.

In starvation the energy of the body has to be yielded not only by the muscle, comparatively poor in nucleins, but by the metabolism of the constituents of the body (liver, spleen, etc.) which are richer in these substances. It is probably for this reason that the excretion of phosphorus does not fall proportionately to the excretion of nitrogen and that thus the quotient in the excretion increases. But just as the nitrogen is economised in starvation so is the phosphorus. During the first few days of the fast in Succi's case the excretion of phosphorus was about 0·9 grm., while after 11 days it fell to about 0·4 grm.[1]

That the alteration in the quotient indicates merely an increased metabolism of the bones I do not think has been definitely proved.

What the source of the fatty acids is in starvation has not so far been established. That they may be derived from the fats of the body seems to be indicated by the phenomena of phosphorus poisoning[2], and by the action of phloridzin on the starving animal[3].

Whether they are also derived from the sugar, which is certainly formed in the liver from proteids during starvation, has not been determined.

The action of the various essential constituents of the diet, fats, carbohydrates, and proteids on the amount of fat in the liver had next to be investigated.

II. Influence of Food.

A. *Fats.*

As already indicated, while the liver is upon the direct path of absorption of proteids and carbohydrates, it is entirely off the path of the absorption of fats. That the fats are, practically, entirely absorbed by the lymphatics of the intestine, is most clearly shown by Munk and Rosenstein[4]. The results obtained by Drossdorf[5] tending to show that the portal blood becomes rich in fats during digestion cannot be accepted[6].

But, though the liver is no more upon the path of absorption of

[1] Luciani. *Das Hungern*, 153. [2] Leo. *Loc. cit.* [3] *Loc. cit.* [4] *Loc. cit.*
[5] *Zeitsch. f. phys. Chem.* I. 233. 1877—78.
[6] Plügge. *Zeitsch. f. Biol.* XII. 1876.

fats than the other tissues of the body, it may nevertheless, like certain of these tissues, be a storage place for the fats absorbed.

In investigating this question, as in investigating the storage of fats generally in the body, the fact that the epithelium of the intestine has the power of selecting, at least to a certain extent, the fatty acids corresponding to those of the body[1] must be remembered.

But Rosenfeld[2] has shown that in animals fed on cocoa butter in phloridzin poisoning this special fat does accumulate in the liver.

While this storage of fat in the liver has been stated by various writers to occur in the normal state[3], no experimental evidence as to its extent and duration has, so far as I am aware, been adduced.

On these points the following experiments throw light.

Exp. 47. 7th August. Five kittens of a brood were suckled by their mother for about six weeks. They were then fed for two days on lean beef, finely minced, and water.

Their weights were as follows:

	2nd	4th	6th	7th	8th	9th	10th
A	279	317	345	317	—	—	—
B	337	375	415	380	385	—	—
C	382	415	450	415	415	—	—
D	340	347	400	360	365	340	—
E	417	447	490	445	445	420	395

From 11 a.m. on the 6th to 5.30 p.m. on the 7th they got no food. They then each got 50 c.cm. of cream containing 18·7 % of fats, so that each took about 9 grms. of fat.

A was killed immediately. Scrapings of the liver showed few oil globules in the cells, which stained dark brown with iodine. The stomach contained fluid cream, but the small intestine contained only a little canary-yellow mucus. The large intestine was full of green fæces.

B was kept without food and killed at 9.30 a.m. on the 8th, 16 hours after food. The liver was pale and creamy, and scrapings appeared like an oil emulsion. The cells stained deeply with iodine. The stomach was empty. The small intestine was lined with a milky layer, which contained oil globules. The villi in scrapings showed numerous oil globules and the lacteals were white. The large intestine contained light greenish fæces.

C was kept without food till 6.30 p.m. on the 8th, 26 hours. The liver was pale and creamy, and contained many oil globules. The iodine staining

[1] Frank. *Arch. f.* (*Anat. u.*) *Physiol.* 1894. 297.

[2] *Verhandl. d.* XII. *Congress. f. innere Med.* 1893.

[3] Kölliker. *Verhandl. d. phys. med. Gesellsch. Würzburg*, VII. 174.

was much less marked than in *B*. The stomach was empty. The small intestine contained only some yellow mucus with a few oil globules. The villi showed no oil globules and the lacteals were not white. The large intestine contained slightly green substance like curded milk.

D was kept without food till 5 p.m. on the 9th, 48 hours. The liver was less pale than in *B* and *C*. The cells contained numerous oil globules, and stained only slightly with iodine. The stomach was empty. The small intestine contained some yellow mucus. Scrapings showed no oil globules either in intestinal contents or in cells. The large intestine contained green fæces.

E was kept without food till 4.30 p.m. on the 10th, 72 hours. The liver was less pale than *B*, *C*, or *D*. Only a few oil globules were seen in the cells. With iodine there was hardly any staining. The stomach was empty. The upper part of the small intestine was empty. The lower part contained some yellow mucus without oil globules. The large intestine contained some pale green fæces.

The bodies of *B*, *C*, and *E* were deprived of the stomach and intestine, and placed in methylated spirit for a week. They were then extracted as in Exp. 42, and the ether extract saponified and the fatty acids separated.

Bodies.

	Weight	Ether extract		Fatty acids		
		Actual	% of body	Actual	% of body	% of ether extract
B	385	4·282	1·11	2·059	0·534	48·0
C	415	12·492	3·00	6·588	1·58	52·7
E	395	6·593	1·67	2·687	0·76	40·8

Livers.

	Weight of liver	Solids	% of ether extract	Fatty acids			
				% of liver	% of ether extract	% of solids	Melting point
A	13·0	25·0	3·57	1·71	47·6	6·84	45° C.
B	16·6	23·4	9·09	6·23	68·5	26·6	34
C	12·5	21·9	8·68	5·97	68·8	27·2	34
D	10·0	20·4	8·94	6·35	71·0	31·0	34
E	11·7	22·6	4·39	2·25	51·5	9·9	41

Melting point of fatty acids of Cream 23° C.
" " " Body 26° C.

Exp. 54. Three rats of the same litter were starved for 24 hours. On the evening of 2/9/94 *B* received 10 grms. of beef fat, *C* received 7 grms. of sugar

with 14 grms. of starch, while *A* was left without food. In the morning *A* was found dead. *B* and *C* were killed at 12 noon.

	Weight of liver	Ether extract % of liver	Fatty acids % of liver	Fatty acids % of ether extract
A	1·6	3·56	1·69	47·3
B	2·0	5·85	3·55	60·6
C	4·0	3·42	1·52	44·5

Exp. 61. Six half-grown rats which had been without food for 12 hours were procured on 8/1/95.

A and *B* were killed at 10 p.m. The stomachs were empty.

C, *D*, *E*, and *F* were put in separate cages, and each got 50 grms. of hard-boiled white of egg.

C was killed at 9 a.m. on the 9th. It had eaten about 30 grms. of the egg, and the stomach was full of egg.

D was killed at 4 p.m. on the 9th. It had eaten about 40 grms. of the egg, and the stomach was full of egg.

E was killed at 9.40 a.m. on the 10th. It had taken about 40 grms. of egg, and the stomach contained egg.

F ate only a very small quantity of egg white, and on the 10th it was fed on ox fat, which it took readily. It was killed at 5.50 p.m. on the 11th. The stomach was full of fat.

Condition	Weight of liver	% of solids less ether extract	Ether extract % of liver	Fatty acids % of liver	Fatty acids % of ether extract	Melting point
A Starved 12 hrs	2·6	24·1	4·77	2·78	58·4	41° C
B "	2·5	24·6	4·72			
C 8 hrs on proteids	5·6	22·3	4·50			
D 15 "	5·2	23·6	4·15	2·34	55·5	41
E 24 "	3·4	24·8	3·85			
F 24 hrs on fats	5·5	23·1	5·94	3·67	61·4	40

Exp. 38. Three young rabbits of a brood, procured on June 7th and starved to June 9th.

Weights:	9th	10th	11th	12th
A	565	—	—	—
B	680	—	—	—
C	715	—	822	780

A was killed at 1.40 p.m. on 9th.

B and *C* were fed on milk from morning of 9th.

B was killed at 3 p.m. on 10th.

C was killed at 12 noon on 12th.

	Condition	Weight of liver	% of solids less ether extract	Ether extract % of liver	Fatty acids: % of liver	Fatty acids: % of ether extract
A	Starved 48 hrs	21·7	21·7	4·93	1·97	40
B	24 hrs on milk	33·0		6·03	3·67	60·8
C	72 „	25·2	18·0	5·86	3·22	55

Exp. 36. A brood of four young rabbits was procured on June 4th.

Weights:	4th	6th	9th	11th	12th
A	624	690	700	785	715
B	474	470	465	467	435
C	607	600	660	625	660
D	498	565	612	649	655

A and *B* were fed on cabbage leaves, oats and bran. *C* and *D* were fed on milk.

All were killed on the 12th. The stomachs of *A* and *B* contained the usual vegetable residue and no oil globules. The stomachs of *C* and *D* contained straw and milk. Scrapings of the liver stained with iodine showed the greatest amount of glycogen in *D*, and the next greatest amount in *C*. In *B* there was hardly any.

	Condition	Weight of liver	% of solids less ether extract	Ether extract % of liver	Fatty acids: % of liver	Fatty acids: % of ether extract
A	Vegetable	24·2	21·58	4·51	2·81	63·2
B	„	10·6	21·46	4·31		
C	Milk	17·9	21·59	5·02	3·34	65·3
D	„	26·1	21·00	4·904		

Exp. 75. Four kittens of a brood, born in the laboratory, were suckled by the mother till May 23rd, when they were fed on bread and milk. No food was given after the evening of the 26th. On the 28th they were fed on raw white fish, which they took greedily.

Weights:	28th	29th	30th
A	417	—	—
B	489	465	430
C	555	505	465
D	457	420	400

A was killed at 3.30 on the 28th. The liver was pale; the stomach full of fish; the small intestine empty; the large intestine full of green fæces. The serum was clear. Liver weighed 16·6 grms., of this 5 grms. taken for glycogen, 11·5 taken for fat.

B got no food and was killed at 12 noon on the 30th (48 hours after food). The liver was darker in colour. The stomach and small intestine were empty. Some green fæces in the large intestine. Liver weighed 13·5 grms., of this 3 grms. were taken for glycogen, 10·5 grms. were taken for fats.

C got no food and was killed at 9.30 p.m. on the 30th (55½ hours after food). The stomach and small intestine were empty. In the large intestine were some green fæces. The serum was clear. The liver was dark in colour. Liver weighed 13·7 grms., of which 3·2 grms. were taken for glycogen, 10·5 grms. were taken for fats.

D got no food till 4 p.m. on the 30th, when it was fed on thick cream, of which it took a large quantity. It was killed at 12.30 on the 31st. The stomach contained an oily grey fluid, with oil globules. In the small intestine was a white layer with fine oil globules. The lacteals and thoracic duct were white. The serum was very milky. The liver was large and of a pale cream colour. Liver weighed 19·5 grms., of this 3·5 grms. were taken for glycogen, 16·0 grms. were taken for fats.

				Ether extract			Fatty acids			
	Weight of liver	% of solids	% of solids less ether extract and glycogen	% of liver	% of solids	% of proteids[1]	% of liver	% of solids	% of proteids	% of ether extract
A	16·6	28·1	17·9	5·09	18·1	28·4	2·75	9·7	15·3	54
B	13·5	28·3	23·3	5·05	17·8	21·0	2·90	10·2	12·4	58
C	13·7	28·1	23·4	4·71	16·7	20·0	2·78	9·89	11·8	59
D	19·5	32·3	15·6	11·9	36·8	74·3	10·6	31·0	67·9	89·5

	Lecithin				Glycogen		
	% of liver	% of solids	% of proteids	% of ether extract	% of liver	% of solids	% of proteids
A	2·57	9·1	14·2	50	4·3	15·3	24·0
B	3·04	10·3	15·1	60	0·0	0·0	0·0
C	3·31	11·7	13·0	70	0·0	0·0	0·0
D	1·72	5·3	11·0	14·4	5·26	16·2	33·7

Exp. 78. A young kitten was fed on cream for 10 days. It was killed on June 7th. The body was thin, but there was an abundance of mesenteric fat. The stomach contained clotted cream, and the small intestine a white

[1] The solids less ether extract and glycogen are taken as proteids.

material. The lacteals were white. The serum was very milky. The liver was very large and pale, and weighed 25·6 grms.

		Ether extract	Fatty acids		Lecithin	
Weight of liver	% of solids less eth. extract	% of liver	% of liver	% of ether extract	% of liver	% of ether extract
25·6	14·5	40·2	36·8	91·1	1·42	3·53

These experiments prove that an excess of fat taken in the food is largely stored in the liver cells, from which (Exp. 47) it is gradually got rid of, but only after about 70 hours.

As already noticed by Perls, it is probable that this power of accumulating fat in the liver varies in different animals. He states that fatty infiltration is not easily produced, even by phosphorus poisoning in the pig's liver. In the only observation I have made on the pig's liver—taken from an animal well fattened—the ether extract amounted to only 4·66 per cent., while in the liver of a sheep killed on the same day it was 9·71. In the liver of some fish, *e.g.* the cod and ling fats accumulate to an enormous extent, while in the liver of such fish as the salmon they do not occur in large amounts.

B. *Carbohydrates.*

The relationship of the carbohydrate of the food to the formation of fats in the liver is of very special interest.

It has been clearly demonstrated that in the animal body carbohydrates are a source of fat.

It is also well known that the liver has the power of arresting and storing carbohydrates as glycogen. That this glycogen is in part at least converted to glucose, and in this form sent to the tissues, has been demonstrated beyond doubt by various observers[1].

But the possibility that glycogen is also a source of fat in the liver has to be considered. It has been suggested that the carbohydrates of the liver may be converted to fat and in this form sent to the organism. The theory is without experimental basis, and so far no evidence of the formation in the liver of fats from carbohydrates has been adduced, though Voit, as already stated, has suggested that in the liver such a transformation may occur. The fact that the food used in bringing about the accumulation of fat in the liver of Strassburg geese

[1] See *Edin. Med. Journal*, Dec. 1894.

is largely maize, which is chiefly composed of carbohydrates, favours this view.

The formation of fats from carbohydrates is according to Gautier[1] indicated by the greatly increased excretion of carbon dioxide without an increase in the oxygen taken up[2] after the administration of carbohydrates. According to Gautier the reaction may be represented as follows:

$$13C_6H_{12}O_6 = C_{55}H_{104}O_6 + 23CO_2 + 26H_2O.$$

Although not believing that a process so complex as the conversion of the carbohydrate to the fatty molecule can occur in this simple manner, the observations of Richet and Hanriot undoubtedly favour the view that in the metabolism of the animal body, carbohydrates are converted to fats with considerable rapidity.

The statement is generally made on the authority of Hoppe-Seyler[3] that the liver fats increase after each digestion and thus, to a certain extent, run parallel with the liver glycogen. At p. 280 he says, "The formation of fat in the normal liver is undoubtedly connected with the formation of glycogen, though it may occur later."

Is there then any relationship between the amount of fat and the amount of glycogen in the liver? On this subject the following series of observations were made by Dr J. J. Douglas. The "fat" is here the ether extract of the liver extracted as above described. The glycogen was extracted by the method of Brücke.

Relationship of "Fats" to Glycogen of Liver.

Exp.	Animal	% of glycogen	Ether extract	Remarks
1	Rabbit	7·604	1·15	Fed on carrots
2	"	0·304	2·98	Starved for 2 days
3	"	5·741	2·66	Fed on turnips, bran and oats
4	"	trace	3·02	Starved for 2 days
5	"	1·433	3·98	Fed on turnips, bran and oats
6	"	2·95	1·81	Fed on mash of lentils
7	"	2·299	3·00	Fed on gluten and bran for 6 days
8	Kitten	6·676	2·63	Suckling, 6 days old
9	"	4·062	3·71	Same brood as 8; starved for 24 hours

The next set of observations are from my own series of experiments. Except in Exp. XIII. the carbohydrates were estimated as glucose by

[1] *Biol. Centralb.* XIV. 417. 1894.

[2] Richet and Hanriot. *C.R.* CXIV. 371. 1892.

[3] *Physiologische Chemie*, 717.

extracting the liver by repeated boiling in large quantities of water, then precipitating the proteids by boiling with acetate of soda and acetic acid and converting the carbohydrates to glucose by boiling with dilute sulphuric acid. The glucose was titrated by Fehling's method.

Exp.	Animal	% of glycogen as glucose	Ether extract	Remarks
II	Sheep	0·866	9·78	From slaughter-house
V	Ling	0·960	66·954	
VI	Cat	2·2	5·12	Pregnant
			5·07	
XII	Kitten	1·02	3·86	Suckled
XVII	Rabbit	5·6	6·202	Suckled
XIII	,,	*A* 5·62	4·20	*A* Before digestion in 0·75 % NaCl: at 40° C. for 4 hours
		B 2·22	4·86	*B* After digestion
IX	Child	2·702	3·45	(Full time)
XXVIII	Woman	6·7	7·23	Diabetic

The tables clearly show that there is no truth in the statement that the amount of fat and glycogen in the liver in any way correspond to one another.

But this has no bearing on the question of whether fats are formed from carbohydrates in the liver. For the solution of this question the following experiments on feeding animals on a diet rich in carbohydrates were performed.

Exp. 27. Four young rabbits of a brood were on April 10th put on the following diets.

A and *B* got oats and water. *C* and *D* got oats and water with about 20 grms. of starch per diem carefully mixed with the oats.

Weights:	10th	12th	14th	15th
A	1094	1100	1142	1127
B	976	970	928	925
C	1095	1115	1120	1080
D	825	850	848	852

B developed mange, lost weight and was rejected. The others were killed on the 15th.

	Weight of liver	Ether extract % of liver	Glycogen % of liver	Solids less ether extract % of liver
A	52·5	3·94	3·522	25·9
C	41·8	4·19	4·02	24·6
D	34·7	4·31	4·33	24·0

Exp. 14. Five pigeons which had been kept together were procured on 20/1/94. *A* was at once killed. *B* and *C* were kept without food, but with supply of water until 3 p.m. on the 24th, about 96 hrs. The crops were empty and the gizzards contained only a few stones.

D and *E* were fed on peas which contain:

Proteids about 22 %,

Carbohydrates about 55 %.

They were killed on the 27th.

F and *G* were fed on rice which contains:

Proteids about 7 %,

Carbohydrates about 77 %.

They were killed on the 27th.

Weights:	20th	22nd	23rd	24th	27th
A	—	—	—	—	—
B	—	286	263	257	—
C	—	222	205	187	—
D	—	432	397	412	415
E	—	332	317	312	334
F	—	282	264	262	249
G	—	282	277	234	255

	Weight of liver	% of dry solids less ether extract	Ether extract % of liver	Ether extract % of dry solids
A	4·5	25·58	5·82	18·67
B	3·4	20·6	3·00	14·5
C	3·0	29·7	5·18	14·87
D	8·2	25·5	5·44	17·58
E	4·7	26·2	5·44	17·19
F	5·4	20·6	[1] + 6·27	+ 20·6
G	4·8	25·6	8·53	25·6

Exp. 29. Four young white rabbits of a brood were procured on April 13th. On April 18th the following diets were started. *A* and *B* got green food with 10 grms. of starch per diem. *C* and *D* got green food with 10 grms. dried white of egg per diem.

Weights:	15th	18th	20th	22nd	23rd	24th
A	382	417	442	414	425	427
B	293	330	353	340	350	335
C	357	357	371	362	375	350
D	293	345	350	338	317	300

[1] Some loss.

extracting the liver by repeated boiling in large quantities of water, then precipitating the proteids by boiling with acetate of soda and acetic acid and converting the carbohydrates to glucose by boiling with dilute sulphuric acid. The glucose was titrated by Fehling's method.

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XVII	Rabbit	5·6	6·202	Suckled
XIII	,,	*A* 5·62	4·20	*A* Before digestion in 0·75 % NaCl: at 40° C. for 4 hours
		B 2·22	4·86	*B* After digestion
IX	Child	2·702	3·45	(Full time)
XXVIII	Woman	6·7	7·23	Diabetic

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D	8·2	25·5	5·44	17·58
E	4·7	26·2	5·44	17·19
F	5·4	20·6	[1] + 6·27	+ 20·6
G	4·8	25·6	8·53	25·6

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Weights:	15th	18th	20th	22nd	23rd	24th
A	382	417	442	414	425	427
B	293	330	353	340	350	335
C	357	357	371	362	375	350
D	293	345	350	338	317	300

[1] Some loss.

These diets were continued till the 24th. On the 25th all were killed at 10.30 a.m. *A* and *B* were strong and well. *C* and *D* were weak and listless.

	Weight of liver	% of solids less ether extract	Ether extract % of liver	Ether extract % of dry solids	Fatty acids % of ether extract
A	15·3	23·0	5·43	19·08	64·2
B	10·8	22·7	5·46	19·3	
C	9·0	23·9	4·87	16·9	64·1
D	8·0	23·7	4·72	16·6	

While Exps. 14 and 29 seem to indicate that a diet rich in carbohydrates causes an increase in the liver fats, this is not so clearly indicated by Exp. 27. But here the amount of carbohydrates given proved barely sufficient to raise the amount of glycogen in the liver, and thus could hardly be expected to have raised the amount of fats.

But such observations are not well calculated to elucidate the question of whether fats are formed from carbohydrates. It is known that carbohydrates are in the first instance stored in the liver as glycogen. If fats are formed from carbohydrates they must be produced from the hepatic glycogen, and therefore any increase in the hepatic fat due to changes in the carbohydrates should make itself manifest at the period when hepatic glycogen is disappearing. Prausnitz[1] has shown that in hens, at least, the glycogen accumulates during the 12 or 24 hours following a meal and then steadily diminishes till, at the end of 48 hours, it has reached a very small amount. That the disappearance occurs in about the same time in rabbits has been for long known. Two days' starvation leaves the liver nearly free of glycogen.

Does an increase of fats occur as the glycogen disappears? The following experiments elucidate this point.

Exp. 57. Three rabbits were kept in the same cage on a diet of turnips and oats from October 25th to November 27th.

Weights:	Oct. 25	Nov. 2	Nov. 26	Nov. 27
A	1550	1770	1849	—
B	1825	1967	2074	1950
C	1549	1670	1749	1640

[1] *Zeitsch. f. Biol.* XXVI. 377. 1890.

A was killed at 1 p.m. on Nov. 26th.
B was killed at 3.30 p.m. on Nov. 27th.
C was killed at 1.15 p.m. on Nov. 29th.
B and *C* had no food from 1 p.m. on Nov. 26th.

Condition	Weight of liver	% of solids less eth. ext. and glycogen	Ether extract	Fatty acids: % of liver	Fatty acids: % of ether extract	Fatty acids: % of solids	Fatty acids: Melting point	Glycogen
A Taking food	54	19·4	3·94	2·17	55	11·2	33° C.	2·68
B 26·5 hours after food	44	16·9	5·76	3·34	57·9	19·7	35	2·93
C 72 hours after food	33	23·7	6·59	4·39	66·6	18·5	35	0·65

Exp. 58. Three small rabbits of a brood were put on a diet of turnips and green food from Dec. 3rd to Dec. 12th.

Weights:	4th	6th	11th	12th	13th	14th
A	374	420	435	420	—	—
B	420	475	510	490	485	—
C	511	555	595	580	530	490

A was killed at 4.15 p.m. on Dec. 12th.
B was killed at 4.30 p.m. on Dec. 13th.
C was killed at 4 p.m. on Dec. 14th.

Condition	Weight of liver	% of solids less eth. ext. and glycogen	Ether extract	Fatty acids: % of liver	Fatty acids: % of ether extract	Fatty acids: % of dry solids	Fatty acids: Melting point	Glycogen
A Taking food	21·0	18·55	3·12	2·07	53·9	10·5	36° C.	5·65
B 24 hours after food	22·2	19·70	2·55	1·60	52·1	8·1		3·00
C 43 hours after food	20·7	23·05	6·27	5·15	60·6	22·3	36	0·15

These experiments show a marked increase in the fatty acids in the liver at the period when the glycogen is disappearing.

That it is an actual increase is shown by the fact that there is an actual increase of fats in relationship to the solids deprived of glycogen and fats.

Is this increase due to an increased formation of fat from glycogen in the liver, or is it due to a transportation of fat to the liver as the glycogen disappears? Were it derived from the glycogen we should expect the accumulation of fat to be most active *during* the rapid

disappearance of glycogen and not some time after the glycogen had reached so low a level. But there can be no doubt that as the glycogen disappears the fat increases.

The evidence of this increase being due to a transport from the adipose tissue of the rest of the body must first be considered.

That fats are transported to the liver and stored there is shown by Exp. 47. But these are food fats, and the proof that the body fats can be so transported is by no means so satisfactory. Nasse, as already pointed out, admits that such a transport has only been proved in phosphorus poisoning, though he states that in starvation the serum may become milky from the taking up of the fats from the body. This of course does not prove that they are transported to the liver.

In the following experiment the blood serum was carefully examined and no milkiness appeared though the liver fats rose from 1·75 to 5·26 % of the liver substance.

Exp. 63. Three rabbits were fed on turnips from the 5th to 7th February.

A was killed on the 7th. The blood serum was clear and free from fats.

B was kept without food till 5 p.m. on the 8th (36 hours) and was then killed. The blood serum was perfectly clear.

C was kept without food till 11.10 a.m. on the 9th (52 hours) and was then killed. The blood serum was perfectly clear.

	Weight of liver	% of dry solids less ether extract	Fatty acids	
			% of liver	% of solids
A	45·6	19·3	1·75	9·07
B	48·3	15·2	2·36	15·5
C	38·0	14·9	5·26	34·9

The fact that the liver fats are characterised by their high percentage of stearic and palmitic acid, and consequently by their high melting point as compared with the body fats, affords a further means of investigating if the increase in the liver fats is due to a transport or not.

If the increase is due to such a transport then there should be a lowering of the melting point of the liver fats as these increase, just as in Exp. 47, after administration of cream the fats which accumulated in the liver had a lower melting point.

The melting point of the fatty acids of the liver and of the body generally has been considered on p. 179. In the rabbit,

Fatty acids of liver melt at about 36° C.

Fatty acids of body melt at about 24° to 25° C.

In Exps. 57 and 58 the melting point of the fatty acids of the liver remained nearly uniform throughout the whole period. This fact, along with the persistent absence of fat from the serum, militates against the view that a transport has simply taken place from the adipose tissues to the liver, and tends to the conclusion that fatty acids are produced in the liver by the metabolism of glycogen.

In coming to such a conclusion one is forced to consider the very curious results obtained by Rosenfeld on the fatty infiltration produced by phloridzin poisoning[1]. He showed that the administration of phloridzin to the starving dog and still more to the dog fed on fats leads to a great accumulation of fat in the liver. But if the animal is fed on sugar or lean beef this storage of fat is greatly diminished, while glycogen is accumulated. What the explanation of this is is not clear. Possibly the increased hepatic metabolism induced by these substances may lead to an increased conversion of fat in the liver and may thus prevent its accumulation. At first sight such an observation may seem to oppose the view that fats are formed from glycogen, but in reality it has no bearing upon it. Rosenfeld's observations do not extend to the fate of the glycogen thus stored in the liver.

C. *Proteids.*

The question whether fats are formed from proteids is one which, on account of the recent investigations and arguments of Pflüger[2], is again exciting the attention of physiologists.

The experiments of Bauer[3] on phosphorus poisoning, of Hofmann[4] on the formation of fat in maggots; of Pettenkofer and Voit[5] on fat formation in dogs on a proteid diet; the fatty changes in the ripening of cheese[6] and the occurrence of fatty degeneration in pathological conditions, seem at first sight to indicate that fats are formed in the animal body from proteids.

But according to Pflüger's observations this fat formation in cheese and in Hofmann's experiments, is due to the action of fungi and not of animal cells. While the careful examination to which he subjects Pettenkofer and Voit's well-known experiments shows that the source of the fats may have been the fats and carbohydrates

[1] *Verhandl. des Congres. f. innere Med. Zwölften Con.* 359. 1893.
[2] *Pflüger's Arch.* 52, 1 and 239. 1892.
[3] *Zeitsch. f. Biol.* XIV. 527. 1878.
[4] *Zeitsch. f. Biol.* VIII. 159. 1872.
[5] *Zeitsch. f. Biol.* VII. 433. 1871; IX. 1. 1873.
[6] *Pflüger's Arch.* LIV. 484. 1893.

of the food. The experiments of Bauer[1] he does not consider, but these have been severely criticised by Falck, who maintains that the experimental methods are unsatisfactory. But even supposing that the results obtained by Bauer are accurate the conclusions based on these results are still open to question.

According to his observations on starving dogs poisoned with phosphorus, the amount of fat in muscle, heart muscle and liver is markedly increased. The excretion of nitrogen is enormously increased, while the excretion of carbonic acid is diminished. From this he concludes that the proteid molecule is split up, the nitrogenous part being excreted while the carbonaceous part is retained as fat.

But may this not merely indicate—what indeed he confesses must occur[1]—a diminished consumption of fat without necessarily an increased production?

"We find in phosphorus poisoning two alterations in the metabolism unconnected with each other; first an increased decomposition of albumin, and second a lessened taking up of oxygen and lessened fat destruction."

The argument that because in 20 days' starvation visible fat has disappeared from the tissues is of no importance because the experiments of Hofmann[2] on dogs have shown that fat may be still present at the end of almost 30 days' starvation.

The pathological change of fatty degeneration is always considered a strong argument in favour of the proteid of the cell being a source of the fats. But the more the subject is investigated the less conclusive becomes the evidence on this point.

Perls[3] gives the following results of his investigations on the amount of fat, *i.e.* ether extract, in the liver.

	% of fats	% of other solids	% of total solids
Normal livers (5 analyses)	3·8	19·1	22·9
Fatty infiltration (6 analyses)	25·8	15·6	41·4
Fatty degeneration (2 analyses)	8·1	12·6	20·7

He therefore concludes that fatty degeneration may readily be distinguished from fatty infiltration by the diminished amount of other solids. His two observations show in fatty degeneration a very moderate increase in the amount of fats, and in no way prove that the increase is due to the production of fats from proteids.

[1] *Zeitsch. f. Biol.* VII. 76. 1871.
[2] *Zeitsch. f. Biol.* VIII. 153. 1872.
[3] *Lehrbuch der allgemeinen Pathologie; zweite Auflage*, 115.

His observations on fatty degeneration of the heart give the following results.

	% of fats
Normal hearts (2 analyses)	1·99
Fatty degeneration (6 analyses)	4·05

This question has been more recently investigated by Krehl[1], who gives the results of a long series of analyses of the myocardium in the state of fatty degeneration and in the normal condition. He finds in 100 parts of dry solids about 12 parts of fat, *i.e.* "ether extract," in the healthy condition. Of this 4·2 to 4·6 % is lecithin.

In pathological conditions the fats vary from 6·5 to 25 %, but only in three cases—one of phosphorus poisoning and the other two of pernicious anæmia—was the amount of fat much above the normal. He points out that hearts giving the recognised microscopic appearances of fatty degeneration may actually have less fat than in the normal condition.

In the evidence derived from fatty degeneration there is nothing to prove the origin of fats from proteid. The accumulation may just as well be explained by a diminished utilization of fats in the tissues.

Pflüger's criticism and the pathological evidence leave us in the position of having to regard the formation of fat directly from the proteid as not proven. The formation of carbohydrate material, glycogen and sugar, from proteid is admitted by all, and the formation of fat from carbohydrates is acknowledged even by Pflüger, so that the possibility of an *indirect* formation of fats from proteids in the animal body must be admitted.

Is there any evidence that the liver forms fats directly from the proteids of the food?

On this point it is not easy to adduce satisfactory experimental evidence, for the well-known increase in the hepatic metabolism, which follows a proteid meal, may lead to an accelerated conversion of fat in the liver, as is indicated by Langley's observations on frogs, and this may mask any increased production from the proteid food.

The following experiments however, so far as they go, seem to be opposed to the view that fats are formed in the liver from proteids.

Experiment 29 (see p. 205).

Exp. 30. Two young rabbits of a brood were procured on April 14th.

[1] *Deutsch. Arch. f. klin. Med.* LI. 416. 1893.

From the 15th to the 24th *A* was fed on bran with 10 grms. of starch per diem. *B* got bran with 10 grms. dried white of egg.

Weights:	15th	19th	21st	23rd	24th
A	465	520	529	575	577
B	445	460	467	492	492

No food was given after the morning of the 24th and both were killed at 11.30 a.m. on the 25th.

Scrapings of the liver of *A* stained with iodine showed much glycogen, while similar scrapings from *B* showed very little staining. The cells were smaller and the nuclei more distinct.

	Weight of liver	% of solids less ether extract	Ether extract	
			% of liver	% of solids
A	18·8	24·07	4·8	16·6
B	15·2	23·5	4·8	16·9

In the former of these two experiments a greater amount of ether extract is shown on a carbohydrate diet than on one in which the excess of carbohydrates is replaced by proteids.

Experiments 61 and 75. (See pp. 199 and 200.)

Exp. 55. Three kittens of different broods were procured on the 17th November, 1894. They were kept on bread and milk till the 25th and after 10 a.m. on that day no food was given till 11.30 a.m. on the 26th. They were then fed on lean horse-flesh.

Weights:	24th	25th	26th	27th	28th
A	495	497	469	457	
B	1797	1802	1620		1525
C	932	920	904		

C took hardly any of the flesh and was killed at 3.10 p.m.

A had eaten 50 grms. of flesh at 1 p.m. on the 26th. It was killed at 12.45 on the 27th.

B had eaten 160 grms. of flesh by 1 p.m. on the 26th. It was kept without food and killed at 2.30 p.m. on the 28th.

	Weight of liver	Solids less ether extract	Ether extract	Fatty acids		Melting point
			% of liver	% of liver	% of ether extract	
C	37	22·9	3·14	1·72	54·7	35°
A	24·5	21·2	3·47	1·92	55·3	37
B	45	24·6	3·68	2·15	58·4	37

These observations show that the addition of an excess of proteid to the food does not lead to an accumulation of fat in the liver.

In Experiment 55 the slight rise in the fatty acids is probably that which we have already shown as occurring with the disappearance of glycogen.

Summary of Conclusions.

Throughout the various parts of the liver there is a uniform distribution of the substances soluble in ether—the so-called "fats."

In animals in the same condition the percentage amount of substances soluble in ether is fairly uniform.

The ether extract varies greatly in amount, but is on an average about 5 % of the liver substance.

After extraction with ether a certain amount of fatty acids remains partly in combination with bases as soaps, partly in other chemical combinations possibly of the nature of nucleins or of lecithin albumins which may be decomposed by hydrochloric acid.

The ether extract of the liver contains from 40 to 90 % of fatty acids. The fatty acids constitute roughly about 3 % of the liver.

The fats of the liver are distinguished by containing a much smaller proportion of oleic acid than the other fats of the body.

Lecithin is a constant constituent of the ether extract of the liver, and its amount is fairly constant, averaging 2·35 % of the liver substance or 10·1 % of the solids of the liver. It holds nearly one half of the fatty acids in combination.

The amount of lecithin varies inversely with the amount of fatty acids.

It is impossible by titrating the ether extract by Hofmann's method to determine the amount of free fatty acids. When the lecithin is large in amount the acidity is great, when small the acidity more nearly approximates to that of the body fats.

The amount of cholesterin is smaller than is usually stated, averaging about 0·039 % of the liver substance in the rabbit and 0·029 % in the cat.

The amount of substances other than fats, lecithin and cholesterin in the ether extract of the liver varies from 4·2 % to 22 % of the extract, being on an average 12·1 %. The proportion is lowest when the amount of fatty acids is highest.

Pigments constitute only a small part of this.

Sulphur may or may not be present in the ether extract of the liver. Its presence is probably due to the extraction of jecorin. Its absence does not indicate the absence of jecorin, but is probably due to the decomposition of this substance in the process of drying. The fatty acid constituent of the jecorin will in any case be extracted.

Fats may be transported to and accumulated in the liver.

Fats may also be produced *in* the liver.

Fats do not undergo the same simple transformation in the liver as glycogen does.

Fats accumulated in the liver disappear either by being carried from the organ or by being metabolised in the organ.

Some of the fatty acids are linked with phosphorus and cholin to form lecithin, and this lecithin is a forerunner of the nucleo-compounds of the body.

The liver thus seems to have the function of utilizing and economising the phosphorus of the body by combining it with fatty acids as a stage in its reconversion to nuclein compounds.

In the case of the usual storage of fat in the liver there is no diminution in the amount after a fast of 56 hours in cats and 96 hours in pigeons.

When an excessive amount of fat has been stored in the liver of cats it is got rid of to a large extent in 68 hours.

The liver maintains a store of fatty acids during inanition.

An excess of fat taken in the food is largely stored in the livers of certain animals, *e.g.* cat and rabbit.

The amount of fat in the liver is not proportionate to the amount of glycogen present.

A diet rich in carbohydrates tends to increase the amount of fat in the liver.

As hepatic glycogen disappears there is an actual increase in the amount of fatty acids in the liver.

These acids have the characteristic high melting point of the acids of the liver.

During the period of the accumulation of fatty acids the blood serum remains perfectly clear.

The evidence thus points to the formation of the fatty acids from the glycogen.

The addition of an excess of proteid to the food does not lead to an accumulation of fat in the liver.

APPENDIX.

Amount of Fatty Acids in Ether Extract of Liver of Various Animals.

Exp.	Species of Animal	Remarks	Ether extract % of liver	Fatty acids % of liver	% of ether extract	Method
12	Kitten	Suckling (2)	3·86	1·58	41·2	Lebedeff
31	,,	Three suckling. *A* killed 3 hours after food, *B* 6 hours, and *C* 22 hours after	6·12	3·9	63·0	K and O[1]
16	,,	Fed on fat and bread and milk	3·16	2·0	82·0	Hoppe-Seyler
32	,,	Four days old. *A* and *B* killed at once. *C* and *D* starved 24 hours	5·27	3·54	67·1	K and O
42	,,	Four suckled. *A* and *B* killed	*A*, *B* 4·32	2·63	55·9	K and O
		10 hours, and *C* 34 hours, and	*C*, *D* 5·70	2·58	45·5	K and O
		D 58 hours after food				
25	,,	Three of same litter suckled	5·75	2·66	46·4	K and O
			6·01	2·76	49·0	K and O
			5·05	2·22	44·0	K and O
47	,,	Five of litter. *A* starved 30 hours	3·57	1·71	47·6	
		B 12 hours after cream	9·09	6·23	68·5	
		C 21 ,, ,,	8·68	5·97	68·8	K and O
		D 45 ,, ,,	8·94	6·35	71·0	
		E 68 ,, ,,	4·39	2·25	51·5	
55	,,	Three. *C* starved	3·14	1·72	54·7	K and O
		A 24 hours after proteid food	3·47	1·92	55·3	
		B 48 ,, ,, ,,	3·68	2·15	58·4	
75	,,	Four of litter. *A*	5·09	2·75	54·0	
		B	5·05	2·90	58·0	K and O
		C	4·71	2·78	59·0	
		D 24 hours after cream	11·9	10·60	89·5	
78		Fed on cream for a week	40·2	36·8	91·1	K and O
				Average	64·6	
		or deducting Exps. 16, 75 *D* and 78—			55·5	per cent.
11	Cat	Very fat. Two parts of liver taken	15·02	8·04	53·6	Lebedeff
			15·54	8·1	52·2	Lebedeff
4	,,		5·89	3·15	53·7	Lebedeff
				Average	53·1	
13	Rabbit	*E* liver just after death	4·20	2·31	69·2	Hoppe-Seyler
		D liver after 4 hours at 40° C. in ·75 % NaCl	4·86	3·52	72·3	
27	,,	*A* and *B* on oats and water	*A* 3·94	2·00	49·2	K and O
		C and *D* on oats, water and starch	*B* 4·56	2·33	51·2	
			C 4·19	2·19	50·2	
			D 4·81	2·22	50·2	

[1] Modification of Kossel and Obermüller's method, p. 174.

29	Rabbit	Mixed ether extract of 4 rabbits, divided into two equal parts	5·09 5·09	3·22 3·22	64·2 64·2	K and O
36	„	*A* and *B* fed on green food, oats and bran	*A* 4·51 *B* 4·31	2·81	63·2	K and O
		C and *D* fed on milk	*C* 5·02 *D* 4·90	3·34	65·3	
38	„	*A* starved	4·93	1·97	40·0	K and O
		B milk for 24 hours	6·03	3·67	60·8	
		C milk for 72 hours	5·86	3·22	55·0	
43	„	Young suckled	5·12 3·18	2·42	58	K and O
44	„	Mother of last, emaciated	7·87 7·87	3·25 3·05	41·2 38·2	K and O
48	„	Liver divided into two. *A* placed in spirit at once. *B* kept at 40° for 6 hours in ·75 % NaCl	*A* 3·93 *B* 4·12	2·48 3·43	61·9 62·6	K and O
56	„	*A* normal	4·49	2·9	64·4	K and O
		B small dose of brucine	4·49	2·7	60 0	
57	„	Feeding on turnips	3·94	2·17	55·0	K and O
		24 hours after	5·76	3·34	57·9	
		48 hours after	6·59	4·39	66·6	
58	„	Taking turnips	3·12	2·07	53·9	K and O
		24 hours after	2·55	1·60	52·1	
		48 hours after	6·27	5·15	60·6	
59	„	Very fat	6·54	4·40	67·2	K and O
65	„		4·68	2·82	60·9	K and O
68	„	Four of a litter. *A* and *B* got brucin	6·19	4·32	69·6	K and O
		C and *D* normal	7·39	3·52	47·6	
74	„		3·99	2·45	61·2	K and O
				Average	57·8	
54	Rat	Starved	3·56	1·69	47·3	
		Fed on beef fat	5·85	3·55	60·6	K and O
		Fed on starch and sugar	3·42	1·52	44·5	
61	„	Starved 12 hours	4·77 4·72	2·78	58·4	K and O
		„ „	4·50			
		8 hours after white of egg	4·15	2·34	55·5	
		8 hours after greater quantity white of egg				
		24 hours after white of egg	3·85			
		After 12 hours' feeding on beef fat	5·94	3·67	61·4	
				Average	54·6	
2 *A*	Sheep		7·4	4·9	66·2	Lebedeff
35	Pigeon	Fed on oats	4·69	2·79	60·0	K and O
		Starved for 84 hours	4·9	2·79	56·0	
50	„		4·37 4·46	3·00	67·9	
				Average	61·3	
5	Ling		67·556	39·96	69·1	Lebedeff
7	Skate		11·9	7·88	65·9	Lebedeff

ON THE CHEMICAL COMPOSITION OF ENAMEL.

By CHARLES S. TOMES, M.A., F.R.S.

It might have been thought that the last word had been said upon this subject, seeing that so large number of analyses of enamel have been published, but a comparison of the analyses shows great discrepancies in their results, and especially in the percentages of carbonic acid and of organic matter, the range in which is so wide as to strongly suggest an error somewhere.

As regards the carbonic acid I find about 4 per cent. in elephant's enamel, but the estimation of carbonic acid in the small quantities obtainable is necessarily not very exact.

The question of the organic matter is more interesting and the divergences greater.

Thus to give a few examples, Berzelius[1] gives the organic matter as 2 per cent.; von Bibra[2] as from 3·59 to 5·97 in man, and as 7, 8, 9 and even 10·83 in other mammalia; Hoppe-Seyler[3] as 5·74 (including soluble salts) in the horse, and in the dog as practically none, at least only ·8 per cent. Fremy[4] gives for hippopotamus enamel 10 per cent., including soluble salts; the 10 per cent. he divides up into 7 per cent. soluble in hydrochloric acid, and 3 per cent. insoluble organic matter; and Aeby[5] gives 3·60 as the percentage of organic matter in the cow.

It is expressly stated in some analyses that the organic matter was estimated simply by loss, whilst others appear, from the manner in which the percentages are set out, to have been conducted as analyses of an ash, so that in these also the organic matter was probably estimated by the loss on ignition.

In one point all the analyses agree, in that no water is set down as a constituent of completed enamel.

[1] Berzelius. *Simon's Chemistry*. Sydenham Society.
[2] von Bibra. *Chem. Untersuch. über die Knochen und Zähne*, 1844.
[3] Hoppe-Seyler. *Physiol. Chemie*, 1877–81.
[4] Fremy. *Annal. d. Chemie*, iii. XLIII.
[5] Aeby. *Centralblatt f. medicin. Wissenschaft*, 1873, No. 7.

It is a matter of extreme difficulty to get enamel free from other dental tissues; so difficult is it that very close agreement in results is hardly to be expected, for those animals which have enamel of such thickness that a reasonable quantity can be got generally have it convoluted, so that it can only be separated from adherent tissues by grinding away so much of its surface as to sacrifice a large proportion of the enamel itself.

With the object of arriving at some explanation of the discrepancies, and also of studying its own nature, I endeavoured to estimate the organic matter directly, and soon arrived at the somewhat surprising conclusion that there was none, or not enough to amount to a weighable quantity.

The method at first followed was to dissolve the enamel in dilute nitric acid, adding it by degrees so as to use only just enough to effect solution of the lime salts; this usually left a residue of a few shreds, which on microscopic examination proved to be pieces of dentine, cementum and Nasmyth's membrane, in no case in which the enamel had been carefully prepared amounting to more than ·4 per cent., and generally being far less.

The solution after being filtered was cautiously neutralised with ammonia till it became cloudy, cleared with acetic acid and then tested for proteids.

No precipitate was obtained with tannin, picric acid, nor lead acetate, only the very slightest tinge of yellow with the xantho-proteic reaction, no biuret reaction, and no colour with Millon's reagent. Lest the presence of the lime salts should have masked any of the reactions they were precipitated in the usual way as oxalate and the filtrate again tested with similar negative results: neither absolute alcohol nor saturation with ammonium sulphate gave a precipitate.

As a check experiment a solution was prepared which contained lime phosphate and carbonate in the proportions in which they exist in enamel, and ·25 per cent. of dry collagen obtained from dentine was added. In this mixture the presence of the proteid was established with the utmost plainness, so that the conclusion appeared irresistible that there was less than ·25 per cent. of proteid or proteoid substance in the enamel used.

Lest there should be a body insoluble in acid, some enamel was powdered and digested for twenty-four hours in weak baryta water; on acidifying this with acetic acid a cloudiness was produced which after many hours settled; it was however too little to weigh. A similar

result was obtained with lead acetate, so that the alkaline solution had taken up a trace of mucin, or perhaps as suggested by Professor Michael Foster, of nucleoalbumin.

But the small trace obtained was insufficient for the determination of its exact nature.

Up to this point the enamel of man, the horse and the elephant had been employed, but as they showed no differences, that of the elephant's molar was selected for further experiment, as it is possible to get it in larger quantities.

The dentine and cementum were scaled off it with chisels and all adhering fragments that could be seen removed by filing or grinding; the enamel thus obtained was broken into small pieces and dried for at least six hours at 130° C.

On ignition, which was done in a muffle to secure uniformity of heating, specimens of this enamel were found to lose from 5·8 to 7·5 per cent. of weight; the previous experiments had shown that at all events there was nothing like that amount of organic matter, so it seemed probable that it must be water, an inference strongly suggested by the loss of some samples by their flying violently into pieces after reaching red heat, and by something blowing out between the platinum crucible and its lid, when a lid was used to prevent loss.

Of course the usual correction for loss due to the driving off of carbonic acid from carbonates by moistening the ash with ammonium carbonate was in all cases applied, though as a matter of fact the ash having been weighed both before and after, but little carbonic acid was found to have been driven off in these ignitions.

Portions of enamel were then placed in a glass tube the end of which had been sealed, and the other end then drawn down to a fine tube. On gently raising the end containing the enamel to redness its behaviour could be watched; when it nearly reached redness it began to crepitate and violent disruption of some of the fragments took place, these being projected far up the tube; at the same time a cloud appeared in the cool end of the tube and beads of water condensed therein, sufficient in quantity to coalesce and run down the tube.

The tube being then again weighed was found to have lost but little, but on reheating it, and heating the whole length so as to drive the water out, it was found that the loss was from 4 to 4·5 per cent., or a great part of the total loss found on ignition in a crucible.

It was thus shown visibly that it was water which caused most of the ignition loss, and on repeating the experiment, having converted the

end of the tube into an extemporised calcium chloride tube, the water could be driven out of the enamel, rendered visible, then driven on into the chloride of calcium and absorbed, and the weight found to be nearly constant, so that an ignition with practically no loss of weight was performed.

The ignition in the tubes was instructive in several respects. The violent disruption and projection of fragments of enamel suggested a source of error in ordinary ignitions which may explain some of the discrepancies.

Then also there was a brown stain where the water first condensed which may have been due to some empyreumatic products, and so pointed to the presence of traces of organic matter; but on examining the enamel at the bottom of the tube, though it was seen to be darkened, it was noticed that the darkening was almost wholly confined to the immediate surfaces which had been in contact with dentine or cementum, and that the fractured surfaces of the interior of the enamel remained snowy white. This was a confirmation of the results got by microscopic examination of the insoluble residues, and pointed to the impossibility of getting the enamel really free from portions of other adherent tissue, so that it seems a fair inference that the empyreumatic products were derived from these surfaces and not from the enamel itself. So far then the almost entire absence of organic matter and the presence of a notable quantity of water had been established; it remained to enquire in what form that water existed.

Tribasic calcium phosphate, which is the form in which the lime and phosphoric acid in bone and in dentine are believed to be combined, when prepared by wet processes contains from one to several equivalents of water in combination, and only loses the last of these at bright red heat.

As in the development of the tooth it is laid down in the presence of much water the belief of Aeby, that the extra hardness of enamel was due to the lime salt in it being anhydrous, whereas that in bone and dentine contained combined water, never appeared probable, and my own investigations render it untenable.

As an average analysis of enamel we may take one of von Bibra's series:

$Ca_3P_2O_8$	89·82
$CaCO_3$	4·37
$MgHPO_4$	1·34
Organic Matter	4·27

Now supposing that this 89·82 per cent. of calcium phosphate held in combination one equivalent of water, the percentage of water present would be 4·93, an amount not widely different from the loss set down as "organic matter" in his analysis. Indeed, considering that for the reasons given above, analyses of enamel can hardly ever be absolutely accurate, the coincidence is exceedingly close, and if all the results worked out as well the question that instead of organic matter we should read water, in the analyses of enamel, might be held to be settled.

But unfortunately they do not by any means do so; thus with elephant enamel my own ignitions show losses larger than this, viz. 5·72, 6 and 7·1, and in the horse 7·2 and 7·8, and in man 7·8.

Taking the calcium phosphate at about 90, which seems to be about the average, these ignition losses are too large to represent exactly one equivalent of water, and too small to be two equivalents. And yet the impossibility of getting any satisfactory reaction with tests for proteids or proteoids renders improbable the assumption that there is one equivalent of water, and that the rest is organic matter.

It is pretty evident that the inherent difficulties render more than approximations impossible; thus Hoppe-Seyler gives for horse enamel 95·03 per cent. of salts, whereas von Bibra gives only 92·75, and I find 92·8. Thus Hoppe-Seyler's "organic matter" corresponds in weight very closely with one equivalent of water (it is noted that it was determined from loss, but does not say whether loss on ignition, or the deficiency when the lime and magnesium salts had been separately estimated), but von Bibra's and my own results give a larger loss.

Upon the whole the conclusions that I am led to draw are

(i) That there is very little organic matter in enamel, not enough for quantitative estimation.

(ii) That there is at least one equivalent of water in combination with the calcic phosphate, and this, which is not mentioned in any analysis which I have been able to find, generally has figured as organic matter.

(iii) That there is more water than this in enamel: it may be that from its physical structure it is very hard to dry, though longer drying and a somewhat higher temperature make little difference to it.

Or it may be that there are two equivalents in combination and that the salt is exceedingly hard to decompose absolutely at the temperatures obtained; some of von Bibra's analyses do actually give an

amount of loss attaining to the theoretical quantity required for two equivalents.

Dry dentine loses by ignition some 28 per cent. of its weight, but of this a large amount, somewhere about 7 or 8 per cent., is water, and the resulting 20 per cent. of organic matter retains the form of the dentine and is of very firm consistency, so that had enamel really contained anything like 4 per cent. of organic matter a very appreciable and even coherent residue might have been expected when it was decalcified.

And I have found that the nacreous layer of the shell of Pinna, which has a prismatic structure like an enamel with gigantic prisms, when decalcified leaves a perfectly coherent organic residue which when wet is more bulky than the fragment of shell from which it was derived.

Yet this conspicuous organic residue when dried is found to only amount to 1·3 per cent. of the weight of the shell employed.

The enamel of the elephant's molar must then be considered as an almost inorganic crystalline structure, and so far as my investigations have gone there is no reason to suppose that the enamel of man, the horse and the ox differ materially from it.

This comparative absence of organic matter explains what has always been a difficulty with histologists, namely, the complete disappearance of enamel under the action of acids.

It is usually said that young enamel contains a much larger percentage of organic matter than adult enamel: to ascertain if this be so I procured some detached plates from the hinder end of a forming elephant's molar, which consist of a hollow flattened shell of dentine upon which only a little enamel and no cementum has as yet been deposited.

The enamel at this stage is not very hard, so that it can be scraped off in powder with a knife. Microscopic examination of the scrapings show them to consist of short pieces of enamel prisms, either detached or laterally adherent in groups of four or five prisms.

A solution of this powder in dilute acids gives only a trace of proteid reaction, though tannin gives a very slight precipitate. There is therefore a little more organic matter than in the finished enamel, though ignition gives a loss of 8·5 per cent., and most of this is found by the methods above described to be water, and there is thus also a little more water than in the finished enamel.

Microscopic examination of the ash reveals the prisms unaltered in form though they have acquired a slightly granular appearance and seem to be less highly refractive. Fiercer ignition makes them break

down more completely though the fragments of prism retain their form obstinately.

The slightness of the alteration by ignition also tends to prove that the properties of organic matter must be small.

When this ash is moistened with water and again dried it is not found to have gained weight, but if it be dissolved in dilute acid, precipitated by neutralisation with ammonia, and again dried at a temperature sufficient to drive off the ammonia salts, it is found to have taken up about two equivalents of water.

This hypothesis, strongly held by Hoppe-Seyler, was not exactly consistent with published analyses of enamel, and necessitated the assumption of error in the estimation of the carbonic acid.

This, for the reason given above, is likely enough; yet it seems unsafe to regard the existence of this compound as established unless the analyses bear it out more exactly.

Several interesting considerations arise from these results. In the first place the practical absence of organic matter renders untenable or almost untenable the view strenuously advocated by Heitzman, that the enamel is permeated by a complete protoplasmic reticulum and is so to speak alive, he having gone so far as to state that in caries the protoplasm proliferates and vastly increases.

Then again it seems to favour an excretion theory of enamel development rather than the view that the lime salts are deposited in and harden existing epithelial cells, for it seems difficult to understand so complete a disappearance of the organic matter if it ever was there.

So far as it goes, it also tends to render less probable the view of Hoppe-Seyler that the lime salt is a complex one, analogous to apatite, in which the radicle CO_3 has replaced Fl_2.

Apatite $Ca_{10}Fl_2(PO_4)_6$

Bone salt $Ca_{10}CO_3(PO_4)_6$.

Or differently expressed

Bone salt $3Ca_3(PO_4)_2$, $CaCO_3$

one equivalent of calcium carbonate having combined with three of calcium phosphate.

But that the water which I have found in enamel exists in chemical combination, even though the quantities found in the too small amounts of enamel obtainable are not exactly right, is indicated by its not being given off till a high temperature is reached, and then by its being set free so suddenly as to lead to the disruption of the fragment experimented upon.

THE MAXIMUM MUSCULAR EFFORT OF THE HORSE.

By Veterinary-Captain F. SMITH, F.R.C.V.S.

THE subject of horse traction has given rise to many experiments, principally to determine the most favourable load to be drawn under various conditions of road and pace. Such experiments were of the greatest interest and value, especially in the days of mail coaches, when it became an important matter to determine the heaviest weight a horse could draw at a given pace without shortening its useful life.

The observations I have undertaken are somewhat different; no attempt has been made to ascertain the most generally useful force a horse should exercise in draught, but the enquiry has been directed towards ascertaining the maximum amount of force a horse is capable of exerting at a given moment—in other words, to determine the limit of his power.

Of the previous work on this subject I only know of a few isolated experiments which were not published; they were considered unreliable, as it was found impossible to prevent the horse from jerking the dynamometer in bounding forward to get free. It is obvious that any results obtained by jerking are useless, and for some time I could not see how to overcome the difficulty. It would have been possible to train one or two horses to exert a steady pull on the instrument, but this number would have been insufficient for my purpose.

A horse attached to a dynamometer throws himself into the collar, and bounds forwards when he finds he is unable to move; I did away with this difficulty by not securing the dynamometer to a post or tree, but attaching to it a long rope on which were placed a certain number of men. These men were trained to move forward with the horse in the first instance, and then gradually to put their united weight into the rope, and so resist the animal's progress in a perfectly even manner.

As the horse experienced the resistance he exerted more strength, and either pulled the whole of the men along readily (in which case more were placed on the rope), or was only just able to move the

resistance. This latter was taken as the limit of his power, and the steady pull registered at this moment by the dynamometer was recorded.

All the experiments were conducted on a level surface covered by a thick layer of tan; this afforded a good foothold to the horse and prevented any accident in case he fell.

The muscular force a horse can exert in draught is governed by the weight of its body; other things being equal, a heavy horse will pull more than a light one. In my experiments an attempt is made to determine the proportion which the body weight bears to the limit of power; for this purpose all the horses were weighed.

The total number of observations made was eighty-three, and the number of horses employed was eighty. The observations may be grouped under four heads, the grouping being determined by the spirit in which the horse did its work. For example, a horse is classified as 'excellent' which met the gradually increasing resistance by a gradual increase in force, eventually straining his utmost, sometimes lowering the body until the knees almost touched the ground in order that more weight might be placed in the collar, whilst one which met the resistance by relaxing instead of increasing its efforts, and was only made to pull by dint of stimulation, is classified as 'bad'; between these extremes come 'good' and 'fair.'

The classification under which the horse came was noted at the moment of the experiment, and though I have a few horses in the group 'excellent' which have failed to attain anything out of the ordinary on the dynamometer, I have nothing in the group 'bad' which should have obtained a higher position. As explained above, the group into which a horse fell was not determined by the weight he pulled, but by the spirit in which he pulled it.

In the group 'excellent' there are thirty-seven observations; the average weight of the horses was 1256 lbs., and the average weight pulled was 986 lbs. We may therefore say that a horse exerting itself to the greatest possible extent, cannot exercise a dead pull of more than 78·5% of its body weight.

In the group 'good' there are fifteen observations; the average weight of the horses was 1213 lbs., and the average weight pulled was 942 lbs., or 77·6% of the body weight.

A group of 'fair' pullers ten in number gave an average body weight of 1201 lbs., and an average pull of 848 lbs., or 70·6% of the body weight.

In the group 'bad' some horses are included which I classified as

'indifferent'; the total number forming the group is nineteen, with an average weight of 1225 lbs., and an average pull of 804 lbs., or 65·6% of their body weight.

This enquiry only tells us the greatest effort a horse may exert at a given moment, it does not deal with his useful effective force in draught. This latter is liable to considerable variation, the state of the road and nature of the vehicle being the most important factors.

As a means of comparison with the above results I may say that Brunel[1] considered a force of traction of 100 lbs. to 150 lbs. to be the most suitable for slow work of eight hours per diem; we may take the average of this at 8% of the body weight. During fast draught work the effort is considerably reduced, 40 lbs. per horse was the force of traction employed in the fast mail coaches of years ago, or four per cent. of the body weight, taking the latter at 1000 lbs. Under these conditions a day's work was performed in about fifty minutes, the stage being eight miles.

[1] Youatt. *The Horse.* Art: 'Draught.'

ON THE PASSAGE OF SODIUM IODIDE FROM THE BLOOD TO THE LYMPH, WITH SOME REMARKS ON THE THEORY OF LYMPH FORMATION.

By LAFAYETTE B. MENDEL, Ph.D., *New Haven, Conn., U.S.A.*

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For a long time the formation of the lymph was regarded as a simple process of mechanical filtration out of the blood plasma into the lymph spaces surrounding the capillaries. In his paper "Versuche und Fragen zur Lehre der Lymphbildung[1]," Heidenhain first pointed out that if the demands of various organs for specific substances are considered in connection with the amount of lymph actually formed in the body, we arrive at results which a theory of simple filtration fails to explain. Thus he showed that on this theory, the CaO in the milk excreted daily by a cow would require a daily formation of about 236 litres of lymph in the mammary gland alone, an amount which is entirely out of the question.

A plausible explanation of this and other similar facts is given by diffusion, the substance required by each tissue diffusing from the blood to the lymph; from which it is taken by the tissue, and so its tension in the lymph is reduced practically to zero. According to this view, every organ might, as Heidenhain suggests, be provided with the required blood constituents in somewhat the same way as it is with oxygen.

In his investigations Heidenhain sought to determine whether such an explanation, plausible and possible as it seems, stands in accord with the facts of experiment. His observations on the passage of dissolved substances—sugar and sodium chloride, for example—from the blood to the lymph revealed facts which seemed to contradict a simple physical theory of lymph formation, and suggested that other

[1] R. Heidenhain. *Pflüger's Archiv*, XLIX. 1. 1891.

factors *assist* in this process[1]. Thus, he found that the percentage of sugar and sodium chloride in the lymph may rise above that in the blood. Heidenhain, in consequence, formulated his secretion hypothesis, the essential part of which is that the living capillary wall exercises a motive power on the passage of substances from blood to lymph. Of the hypothesis he says[2]: "Sie unterscheidet sich von der Diffusionshypothese dadurch, dass letztere die Triebkraft für den Uebertritt gelöster Substanzen aus dem Blute in die Lymphe lediglich in quantitativen Unterschieden der chemischen Zusammensetzung beider Flüssigkeiten sucht, während die Secretionshypothese die Triebkraft von den lebenden Capillarwandungen ausgehen lässt."

This hypothesis does not exclude physical factors. Heidenhain has pointed out that diffusion undoubtedly occurs, and that the question at issue is whether diffusion and filtration suffice to explain all the phenomena of the formation of lymph. (*Op. cit.* p. 14.)

Recently W. Cohnstein[3] has advocated a purely physical theory of lymph formation, in which filtration and diffusion are the important factors; and he has attempted to show that the observations of Heidenhain already referred to can be entirely explained by such a theory. Cohnstein's earlier observations have already been discussed in part by Heidenhain[4].

As "transudation" Cohnstein designates the changes that take place when one fluid flows under pressure through a tube of animal membrane which is immersed in a different fluid. That which passes from the interior of the tube through into the external fluid he terms a "transudate." Two factors are obviously instrumental in the formation of the transudate: (1) filtration, due to a difference of pressure between the internal and external fluids, and (2) diffusion, due to the difference between the latter in chemical composition.

In his experiments Cohnstein notes that the calculated concentration of a transudate increases with the pressure under which the external fluid exists. He found in experiments with animal membranes that it was possible by raising the external pressure to make the *concentration* of the transudate 18 times that of the original fluid.

[1] Cf. Heidenhain. *Verhandl. des X. internationalen medic. Congresses zu Berlin*, II. (Abth. II.), 60.

[2] Heidenhain. *Pflüger's Archiv*, XLIX. 14. 1891.

[3] Cohnstein. *Virchow's Archiv*, CXXXV. 514. 1894; also *Pflüger's Archiv*, LIX. 350. 1895; *ibid.* 508; *ibid.* LX. 291. 1895; *ibid.* LXII. 58. 1895.

[4] Heidenhain. *Pflüger's Archiv*, LVI. 632. 1894.

He argues that the fluid external to the capillaries exerts a pressure upon them and that in consequence, the percentage of any given substance in the transudate will be greater than in the blood itself, and thus a small quantity of fluid can transport a large quantity of crystalloidal substances.

Applying this argument to Heidenhain's statement mentioned above, with regard to the CaO in cow's milk, Cohnstein assumes a tenfold or higher concentration of the "parenchyma fluid" formed from the blood plasma, so that 23·6 litres of lymph or less would be sufficient to supply the CaO in the day's milk, an amount below that of the milk which a cow may yield in a day.

Heidenhain has already pointed out the error of the above line of reasoning, viz., that the element of *time* is not taken into account. The requirements of the organs must be satisfied within a *definite period*. I should not take occasion to discuss these statements again, had not Cohnstein failed in his later papers to appreciate the objections. He states[1] that he has specially indicated in his experiments "that the absolute amount of solid substance transferred in unit of time decreases with increasing external pressure." This he considers as exactly the same as saying—in accordance with Heidenhain's objection —"that the time required for the transference of *equal quantities* of solid substance *decreases*[2] with increasing external pressure." The second sentence does not express the same idea as the first, but rather the very opposite. Heidenhain did not emphasize the statement that the time required for the transport of equal quantities of substance *decreases* with the increase of external pressure (as is stated above), but noted that the time necessarily *increases*. It might be assumed that a typographical error had crept into the second sentence quoted above; but on page 360 Cohnstein adds, that by assuming a sufficiently high concentration for the "transudate" of the mammary glands, the daily requirements of the latter might be satisfied by a process of "transudation" (as distinguished from filtration). He fails to appreciate that the *percentage composition* of his "transudate" is no index of the absolute amount of solids afforded. If the external pressure were sufficiently raised, it would be possible to reach a point where the amount of water "transuding" would be equal to zero; the *concentration* of the transudate would equal ∞. But the *absolute amount* of the transudate is proportional to the amount of the latter

[1] *Pflüger's Archiv*, LIX. 359. 1895.

[2] The italics are mine.

that has diffused, and it is obvious that it must be less under the conditions just mentioned, than when an additional part of the fluid is obtained by filtration. The greater the external pressure, the longer the time required to obtain a definite quantity of substance.

There remains to be considered a further fundamental objection to an explanation of the lymph formation on a purely physical basis, viz., the observations of Heidenhain that after injection of sugar or NaCl into the circulation, these substances are regularly found in greater percentages in the lymph than in the blood plasma from which it is derived. It is difficult to explain how a substance can be transported from a less concentrated solution (blood) to a more concentrated one (lymph) by a process of filtration and diffusion alone. Cohnstein has shown[1] that it cannot be due to a possible greater concentration of the salts in the blood of the portal circulation—which Starling regards as the chief source of the lymph flowing from the thoracic duct—and he has repeated the experiments of Heidenhain on dogs. The observations agree, in general, with those of the latter investigator; but the interpretation given to them by Cohnstein leads me to further criticism, inasmuch as the questions raised have a direct bearing upon my own experiments.

The lymph which flows from the thoracic duct at a given moment is obviously derived from the blood at some previous moment. Several minutes may intervene from the time of injecting a readily detectable substance directly into the circulation until it appears in the lymph, and Cohnstein properly observes that it is incorrect to compare the composition of a given portion of lymph with that of blood collected at the same moment. Given a series of analyses of blood and lymph, Cohnstein suggests a method of comparing the data. He assumes that the lymph which has the highest percentage of *e.g.* NaCl will be obtained from the blood which has the highest percentage of NaCl, and he thinks that the lymph of maximal percentage is comparable with the blood of maximal percentage[2].

But since the constituents of the lymph are derived from the blood, it is evident that the latter must yield a portion of its NaCl to the former, in order to bring the lymph to its NaCl-maximum. At the moment that the lymph of maximal NaCl-concentration is formed, it is unavoidable that the blood *should already have experienced its maximal percentage of NaCl.* It is therefore by no means justifiable

[1] *Pflüger's Archiv*, LIX. 377. 1895.
[2] *Pflüger's Archiv*, LIX. 511. 1895.

to compare the maximal NaCl-content of the blood and lymph, on the assumption that the one is derived from the other at a given moment.

Heidenhain's comparisons of the composition of blood and lymph were based upon equal weights of those fluids. Cohnstein has very properly called attention to the fact that inasmuch as the total solids of blood serum and lymph serum are unequal, it is necessary to compare the *volume* percentages for a given substance contained in these fluids. He has calculated the approximate volume percentages for Heidenhain's experiments; the differences between the maximal NaCl-percentages of lymph and blood serum become reduced, but by no means disappear.

Let us turn to the results obtained by Cohnstein after infusion of NaCl and sugar solutions into the blood. In three of six experiments with NaCl and in an equal proportion of sugar experiments, Cohnstein found the "concentration maximum" of the lymph *as high as, or even higher* than that of the blood or blood serum. Moreover, in every one of the experiments recorded, *without exception*, the percentage of sugar (or NaCl) in the lymph remains greater—in some instances markedly greater—than that of the blood, from a certain period to the end of the experiment. In other words, the curve representing the sugar or NaCl content of the lymph remains permanently above that of the blood (or blood serum) after a certain time. The following figures from one of the sugar experiments will further illustrate this.

Exp. VI.[1] Bitch. Weight 22·5 kilos. 12h.13–26'; infusion 70 gr. grape sugar.

	Glycose in water %			Glycose in water %	
Time	Blood	Lymph	Time	Blood	Lymph
12.10		0·128	12.35		1·295
12.21	0·497		12.39	0·604	
12.24·5	1·502		12.40		1·185
12.25·5	1·524		12.44		1·194
12.26		0·870	12.48		1·061
12.28		1·581	12.50	0·566	
12.30	1·141		12.51		1·149
12.31		1·471			

How are these figures to be explained by the transudation hypothesis? Why does not the sugar return to the lymph by diffusion? Cohnstein suggests in another connection that since the paths taken

[1] *Pflüger's Archiv*, LXII. 65. 1895.

by the lymph from various organs to the thoracic duct are of different lengths, the lymph flowing from the duct represents a mixture of lymph portions formed at different times and therefore of unlike concentration. Thus, after NaCl infusion, the brief maximal concentration of the blood causes an increase of NaCl in the lymph, which lasts a relatively long time[1]. In reference to this it is to be noted that in the majority of Cohnstein's curves the content of the injected substance in the lymph is at the end of the experiment *as great as, or greater than* that of the blood *half-an-hour before*. It is scarcely to be assumed that so long an interval should have intervened between the production of the lymph and its discharge from the duct.

As to the time which elapses after the injection of various substances into the circulation until they make their appearance in the lymph of the thoracic duct, Cohnstein has found four or five minutes to intervene after injection of potassium- or sodium ferrocyanide solutions. For small quantities of sodium iodide I have observed even longer periods; for sodium salicylicate Tschirwinski[2] has found from four to seven minutes. In the experiment selected above, the sugar maximum of the lymph (1·581 p.c.) was attained within 2½ *minutes* after that of the blood (1·524 p.c.). We may therefore assume that the lymph maximum was formed at a time even *before* the blood had reached its maximal percentage, *i.e.*, the 1·581 p.c. sugar (lymph) are formed from blood having a content of *less* than 1·524 p.c. sugar.

I believe we are justified, therefore, in saying that the facts and arguments are inadequate to explain *all* the phenomena attending the lymph formation, on the basis of filtration and diffusion (transudation). In the following I shall report the results of some experiments carried out in Professor Heidenhain's laboratory, which are in part a continuation of his former investigations on lymph formation. I have studied the distribution of sodium iodide in the blood and lymph after injection of this salt into the circulation. Heidenhain has shown that sodium iodide belongs to the lymphagogues of the second class, and that these substances have an effect proportional to their osmotic pressures. Given the same quantity of NaCl and NaI, the latter will have the less pronounced lymphagogue effect. Sodium iodide, which L. Asher[3] has already employed in similar experiments, especially

[1] *Pflüger's Archiv*, LX. 294. 1895.
[2] *Centralblatt f. Physiol.* IX. 51. 1895.
[3] *Zeitschr. f. Biologie*, XXIX. 247. 1892.

recommended itself because it is a salt not already present in the body and can be employed without immediately fatal results. For determining the NaI, the following method was adopted after a trial of several others; 10 c.cm. of the given fluid are freed from albuminous matter by the sodium acetate and ferric chloride procedure, and the resulting precipitate is thoroughly extracted with hot water. The filtrates are concentrated to a definite volume and the iodine content determined by titration of weak palladium-HCl solution[1].

The general method of the experiments was the same as that employed by Heidenhain[2] and others. Dogs which had gone without food at least 24 hours were used exclusively. Lymph was collected from the thoracic duct and blood from the left crural artery. The lymphagogue was allowed to flow into the right facial vein from a burette. Protocols follow:

Exp. I. 2. vii. 1895. Dog of 15 kilos. $9^h.21$–$29'$; infusion of 37·5 c.cm. solution containing 30·0 grams NaI = 2 grams NaI per kilo body weight. The iodine reaction[3] is obtained in the lymph, $9^h.25$–$26'$.

Lymph serum[4]			Blood serum	
Time	Amount of lymph collected. c.cm.	Iodine[5] (volume per cent.)	Time	Iodine[5] (volume per cent.)
9.29·5—33	10	0·416	9.30	0·632
9.33 —39·5	20	0·588	9.40	0·466
9.39·5—43	10	0·573	9.50	0·399
9.43 —49·5	10	0·546	10.10	0·371
9.49·5—59·5	12	0·468	10.30	0·373
9.59·5—10.9·5	9	0·465	10.53	0·335
10.9·5 —19·5	6·7	0·401		
10.19·5—29·5	5	—		
10.29·5—51	10	0·376		
10.51 —11.15	6	0·347		

[1] Modified after Neubauer u. Vogel. *Harnanalyse*, 440.

[2] *Pflüger's Archiv*, XLIX. 78, *et seq.* 1891.

[3] After removal of proteids by precipitation with alcohol, and evaporation of latter.

[4] After NaI injection, the lymph invariably grows faintly red after a time. This is also the case with other lymphagogues.

[5] The figures for NaI can readily be obtained from these by calculation.

Exp. II. 9. vii. 1895. Bitch of 17·65 kilos. $9^h.47\frac{1}{2}$–$56\frac{3}{4}'$; infusion of 17·6 c.cm. solution containing 17·6 grams NaI = 1 gram NaI per kilo body weight. The iodine reaction is obtained in the lymph, $9^h.52·5$–$53·5'$.

Lymph serum			Blood serum	
Time	Amount of lymph collected. c.cm.	Iodine (volume per cent.)	Time	Iodine (volume per cent.)
9.55 —10.5	12	0·332	$9.57\frac{3}{4}$	0·425
10.5 —13	12·5	0·363	10.8	0·313
10.13 —23·5	13	0·355	10.28	0·235
10.23·5—37·5	13	0·265	10.48	0·238
10.37·5—58	13·5	0·254	11.8	0·211
10.58 —11.25	13	0·214		

Exp. III. 19. vii. 1895. Dog of 16·5 kilos. $9^h.23$–$28'$; infusion of 25 c.cm. solution containing 25 grams NaI = $1\frac{1}{2}$ grams NaI per kilo body weight. The iodine reaction is obtained in the lymph, $9^h.27$–$28'$.

Lymph serum			Blood serum	
Time	Amount of lymph collected. c.cm.	Iodine (volume per cent.)	Time	Iodine (volume per cent.)
9.29—34	13	0·477	9.29	0·523
9.34—40	13	0·491	9.34	0·425
9.40—47	13	0·437	9.54	0·344
9.47—54	13	0·393	10.14	0·289
9.54—10.3	13	0·355	10.34	0·253
10.3 —14	13	0·355		
10.14—37	17	0·277		

Exp. IV. 25. vii. 1895. Dog of 19·2 kilos. $10^h.4$–$45·5'$; infusion of 39 c.cm. solution containing 39 grams NaI = about 2 grams NaI per kilo body weight. There was a pause of one minute at $10^h.24'$ before taking blood. The iodine reaction is obtained in the lymph, $10^h.10$–$11'$.

Lymph serum			Blood serum	
Time	Amount of lymph collected. c.cm.	Iodine (volume per cent.)	Time	Iodine (volume per cent.)
10.12 —17·5	12·5	0·160	10.25	0·323
10.17·5—25	13·5	0·232	10.46·5	0·477
10.25 —32	13·5	0·313	11.6·5	0·358
10.32 —38·5	13·5	0·358	11.26·5	0·280
10.38·5—44	13·5	0·425		
10.44 —50	13·5	0·462		
10.50 —55·5	13·5	0·507		
10.55·5—11.13	27·0	0·456		
11.13 —11.30	13·5	0·385		

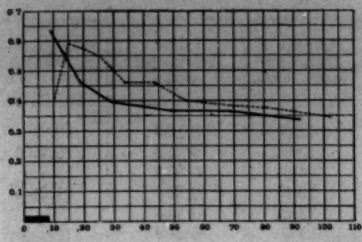
Exp. V. 1. x. 1895. Dog of 17·2 kilos. $9^h.29$–$57·5'$; infusion of 25 c.cm. solution containing 25 grams NaI = about $1\frac{1}{2}$ grams per kilo body weight. There was a pause from $9^h.48'$ to $9^h.49·5'$ before taking blood. The iodine reaction is obtained in the lymph, $9^h.35$–$37'$.

Lymph serum			Blood[1]	
Time	Amount of lymph collected. c.cm.	Iodine (volume per cent.)	Time	Iodine (volume per cent.)
9.37 —49	13	0·270	9.49	0·400
9.49 —58·5	13	0·388	9.58·5	0·443
9.58·5—10.11	13	0·470	10.11	0·347
10.11 —35	13	0·409	10.35	0·296
10.35 —11.5	13	0·332		

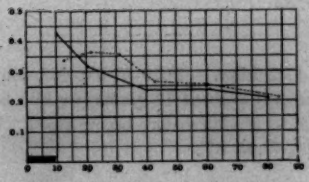
In considering the data presented in the protocols above it is to be remembered that according to Heidenhain's theory injected substances pass from the blood to the lymph by diffusion, but that this process is assisted by the activity of the endothelial cells of the

[1] In this experiment the iodine determinations were made with defibrinated blood. Comparative determinations of the latter and serum from the same sample, in one case, gave results obviously somewhat higher for the serum, yet below that of the corresponding lymph serum.

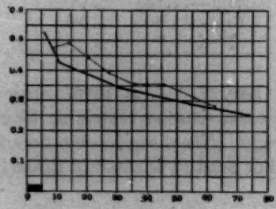
I.



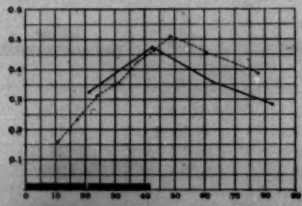
II.



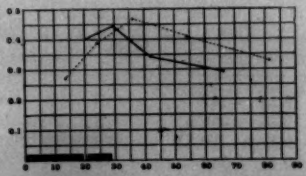
III.



IV.



V.



capillaries[1]. Diffusion will be the more prominent—and "secretion" the less pronounced—the higher the endosmotic tension of the NaI in the blood becomes. The more and quicker NaI is injected, the higher the endosmotic tension. When, on the other hand, the latter is lower, *e.g.* after slow injection, diffusion does not take place so energetically

[1] Cf. above, p. 228, note 1. Also *Pflüger's Archiv*, XLIX. 66. 1891. "Der Uebertritt des Zuckers aus dem Blute in die Lymphe ist nicht bloss der Diffusion überlassen, sondern wird durch eine secretorische Thätigkeit der Capillarwand unterstützt, welche die Schnelligkeit der Entfernung aus dem Blute und das Maass seiner Anhäufung in der Lymphe über die durch die physikalische Diffusion möglichen Grenzen hinaus steigert."

and the coöperation of the capillary walls becomes more evident. This is shown in the diagrams figured above. The abscissas represent time, and the ordinates percentages of iodine found. The time of the infusion is marked by the broadening of the abscissa line. The points of the lymph curves—which are represented as dotted lines in distinction from the blood curves—have been taken at a time midway between the beginning and the end of the period during which each portion was collected. The first blood portion was withdrawn about one minute after the end of the infusion, thus giving the NaI an opportunity to become equally diffused throughout the circulation. Whenever blood was collected during the period of infusion, the latter was interrupted at least one minute prior to the taking of the blood[1].

Diagrams I. II. and III. correspond in general features with those obtained by Heidenhain and by Cohnstein. The observations extend over a longer time, however, and accordingly only the earlier portions of the curves can be compared. Experiments IV. and V. differ from the rest in that the lymphagogue was infused very slowly; the lymph maximum rises above that of the blood, and the lymph curve remains considerably higher above the blood curve than in the other experiments. In Experiments I. II. and III. the sodium iodide was infused with comparative rapidity into the circulation. The osmotic tension of this salt was thereby considerably increased. Accordingly —as explained above—the results approach those which would be expected to follow purely physical processes. Especially after the earlier periods of the experiments, the results obtained might be explained by simple diffusion[2]. On the other hand, in the experiments

[1] In similar experiments of Cohnstein (*loc. cit.*) the blood in which the maximal sugar or NaCl content was determined, was collected *before*, or *immediately at the end* of the injection. A portion of blood thus obtained from an artery, especially after rapid injection of considerable quantities of substance, cannot represent the actual composition of the blood in the capillaries; for it contains the injected substances in a higher concentration, *i.e.* the salt injected into a vein will have passed through the lungs only and not the capillaries of the other tissues, if the blood is withdrawn from an artery *during* the infusion. Thus in his Experiment V. (*Pflüger's Archiv*, LXII. 64) 41 grams of glucose were injected into a dog of 15 kilos, 5 h. 10—13′, *i.e.* in *three* minutes. At 5 h. 12·5′, *i.e. before the end* of the rapid injection, the sugar content of the blood withdrawn was 1·516 %, while *one minute after the end* it was 1·481 %.

[2] Heidenhain has reported two experiments (*Pflüger's Archiv*, XLIX. Figs. XII. and XIII.) and Cohnstein one (*Pflüger's Archiv*, LIX. 522, exp. 1) in which NaCl was infused after ligation of the renal blood vessels. In such experiments the conditions are especially favourable for diffusion, since the osmotic tension of the injected salt in the blood is artificially kept high. I have carried out four experiments in which the kidney secretion was interrupted. In one of these—in which the entire kidneys were ligated—the results

in which the NaI was slowly injected, and in which, therefore, conditions *less favourable for diffusion* existed, the lymph was *richer* in the salt injected than the blood, or blood serum. Even if the lymph curves were so drawn that the maximal points become superimposed on those of the blood curves—and the maxima thus compared in Cohnstein's sense—the lymph curve remains above that of the blood. It is moreover to be remembered that *the actual lymph maxima are even higher* than indicated by the analytical data, since the determinations of NaI were made in portions of lymph collected during several minutes. The figures must therefore represent averages somewhat below the actual maximum occurring at a definite moment. Similar curves to the above were obtained by Heidenhain after slow injection of sugar[1], and in the single experiment of Cohnstein[2] in which the injection of sugar was somewhat slow, his results approach those of Heidenhain and myself. They are not to be explained by a simple hypothesis of filtration and diffusion[3].

Starling has criticised the method of comparison employed by Heidenhain. He says: "I would here merely point out that we do not know how far the composition of the lymph from the thoracic duct represents that of the lymph as it is turned out from the vessels into the tissue spaces. Its composition *must* in fact be determined to

were such as might be explained by simple diffusion, the NaI content of the blood serum and lymph serum being about equal during the greater part of the experiment. In the other three experiments, in which the renal arteries and veins were ligated, the NaI curves for lymph and blood showed marked irregularities. For example, in one case the percentage of I found in the blood serum fell from 0·456 % to 0·296 % and rose again to 0·414 %, while in the lymph serum it gradually increased throughout the experiment; in another, the blood curve showed the same peculiarity as the preceding, while the lymph curve rose, and then soon fell below that of the blood; in a third, the lymph curve alternately rose above and then fell below that for the blood. Analyses showed that these unexpected variations were not due to changes in the water content of the blood and lymph, and I am unable as yet to explain the results. The experiments seem to indicate that the ligation of the renal blood vessels may have other effects—beside the immediate suppression of the urinary secretion—upon the interchange between blood and lymph. Further investigation is desirable.

[1] Cf. for example, Fig. II. *loc. cit.*

[2] Cf. *Pflüger's Archiv*, LXII. 81, Exp. VI. No. 3. Figures from this experiment have already been quoted above.

[3] Hamburger (*Zeitschr. f. Biologie*, XXX. 143. 1893) also found that the composition of the lymph of the horse varies under various conditions in a way that excludes the filtration theory, when the lymph is compared with the blood serum of the same animal. He has also given further objections to the transudation hypothesis (*Du Bois-Reymond's Archiv*, 1895, 363). His work has however been criticised by Cohnstein, *Pflüger's Archiv*, LXII. 77. 1895; and by Leathes. This *Journal*, XIX. 9. 1895.

a great extent by the tissue-cells, and we cannot at once with certainty deduce a secretory activity of the endothelial cells from differences in the composition of the thoracic duct lymph and blood-plasma[1].' So far as this objection applies to my experiments I can think of but two ways in which the lymph could experience an increase of its NaI content in contact with the tissues, viz., (*a*) by obtaining NaI from the tissues; (*b*) by giving up water to the latter. The former is excluded because the tissues contain no NaI. The second possibility is in direct contradiction to the observations of Heidenhain regarding the character of the lymph produced after injection of lymphagogues of the second class. He showed that the water does not arise from the blood, but from the cells, fibres etc. of the tissues[2].

In this connection it is interesting to note that normal lymph has a higher content of NaCl and sugar than the blood collected at the same time—a fact not in accord with the simple transudation hypothesis. The following figures from Cohnstein will illustrate this.

		I.	II.
NaCl[3]	Serum of blood	0·61 %	0·56 %
	Serum of lymph	0·67	0·63
		VII.	VIII.
Sugar[4]	Blood	0·091 %	0·117 %
	Lymph	0·140	0·355

It is obviously impossible that the tissues should give up NaCl to the lymph continuously, as their supply would soon be exhausted.

In conclusion then I do not agree with Cohnstein that the facts brought forward by Heidenhain against the 'transudation' hypothesis can nevertheless be explained by it. The hypothesis does not explain how the maximal content of a substance in the lymph can rise above that of the blood serum, or why in these conditions there is not diffusion in the reverse direction. At the same time there can be no doubt that diffusion, as Heidenhain has repeatedly emphasized, plays an important part in the formation of the lymph. The diffusion and filtration processes fail, however, to account for *all* the phenomena.

I desire to thank Geheimrath Professor Dr. Heidenhain, at whose suggestion I undertook these experiments, for advice and valuable assistance, as well as for the privileges of his laboratory; and Professor Dr. Röhmann for kind suggestions.

[1] Starling. This *Journal*, XVII. 46. 1894.
[2] Heidenhain. *Loc. cit.* 55.
[3] Cohnstein. *Pflüger's Archiv*, LX. 292 et seq.
[4] *Ibid.* LXII. 66 et seq.

A METHOD FOR THE STUDY OF THE INTESTINAL ABSORPTION OF PEPTONE.

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Of the experimental difficulties to be overcome in an investigation of the process of absorption of proteid from the intestine, three, of chemical nature, are at once evident, viz. the complete recovery of the unabsorbed test proteid from a tube that is difficult to wash, its separation from that secreted in the intestinal juice during the period of absorption, and its correct quantitative estimation; to these must be added difficulties of physiological nature arising out of the necessity of ensuring similarity of physiological condition of the gut in all experiments intended for comparison.

It is my intention in this paper to consider the methods of meeting these difficulties and others of subsidiary nature, related to the conduction of such work. The paper naturally divides itself into a Chemical and a Physiological section.

Section I. Chemical.

(*a*) *The separation of the unabsorbed moiety of the test proteid from the proteids of the intestinal juice.*

The proteid used for experiment must, while being of a form ordinarily found in the gut during digestion, be capable of chemical separation from that present in the succus entericus. The most recent analysis of the succus entericus is that by Pregl of juice obtained from a Vella fistula in a lamb. He demonstrates the presence of serum-albumin, globulin, and mucin, and states that albumoses occur in traces, though his proof of their presence is not satisfactory. But even if albumoses are present in the juice of a short piece of gut open to the air at each end, there is no reason for reckoning them as constituents of the normal secretion, in view of the favourable circumstances for a bacterial origin that exist under such conditions.

Obviously then albumoses or peptone or a mixture of these will form a convenient test proteid, since it is possible to separate these substances from serum-albumin, globulin, and mucin.

Bearing in mind the quantities of test proteid used in a series of experiments it is evident that the use of either albumoses or peptone, separately, is out of the question. The processes of separation are long, and the yield of the separated material, especially in the case of peptone prepared by Kühne's method, very small in proportion to the weight of proteid subjected to digestion. We possess however in the commercial "peptones," albumose and peptone mixtures which when suitably treated serve the experimental purpose.

The reagent used for separating the albumose-peptone mixture from the succus proteids, while completely precipitating these, must cause practically no loss of albumose or peptone, and furthermore must not lead to any formation of fresh albumose from the succus proteids during its action.

Obermayer introduced trichloracetic acid as a separator suited to the work, and though he found that "hemi-albumose" was partially precipitated by this reagent, Martin has since shown that at 100° C. albumoses are soluble in solutions of this acid, and Halliburton has recently made use of it in searching for albumoses and peptone in serous effusions.

Martin states that serum-albumin, serum-globulin, fibrinogen, nucleo-albumin, and albuminates are completely precipitated by this acid and that the precipitates are not dissolved on heating to 100°C.

Since mucin is not included in his list I determined its precipitation as follows.

Mucin from Myxine glutinosa (Reid).

·137 gram by the Kjeldahl nitrogen estimation method neutralised 12·43 c.c. $\frac{N}{10}$ sulphuric acid = 12·7 % N.

·104 gram dissolved in ·2 % sodic carbonate solution. Equal volume of 10 % trichloracetic acid added. Filtrate from the precipitate evaporated to dryness and taken up in strong sulphuric acid. On distillation neutralised ·18 c.c. $\frac{N}{10}$ sulphuric acid = ·24 % N,

i.e. 98·12 % of the nitrogen of the substance was precipitated by trichloracetic acid.

Human Saliva.

50 c.c. filtered saliva, evaporated to dryness, and taken up in strong sulphuric acid, on distillation neutralised

$$11{\cdot}53 \text{ c.c. } \frac{N}{10} \text{ sulphuric acid} = {\cdot}032284\ \%\ N.$$

50 c.c. filtered saliva precipitated by equal volume of 10 % trichloracetic acid. Filtrate evaporated to dryness and taken up in strong sulphuric acid, on distillation neutralised ·04 c.c. $\frac{N}{10}$ sulphuric acid = ·000112 % N,

i.e. 99·56 % of the nitrogen of the saliva was precipitated by the trichloracetic acid.

I made in addition one experiment with a sample of serum-albumin.

Serum-Albumin (Grübler). [Ash not calculated.]

·621 gram by the Kjeldahl nitrogen method neutralised 13·7 $\frac{N}{2}$ sulphuric acid = 15·44 % N.

·578 gram dissolved in ·6 % sodic chloride solution, an equal volume of 10 % trichloracetic acid added and heated to 75° C. Filtrate evaporated to dryness and taken up in strong sulphuric acid, neutralised on distillation ·7 c.c. $\frac{N}{10}$ sulphuric acid = ·17 % N,

i.e. 98·9 % of the nitrogen precipitated by the trichloracetic acid.

Reference should here be made to the theory of v. Ott, Popoff and Brinck that albumoses and peptone are converted within the lumen of the gut into serum-albumin before entering the cells for absorption. If there were good evidence of this, my method would be faulty, seeing that there would be a loss of test proteid on treatment with trichloracetic acid as a result of the conversion. I fail to see any basis for their hypothesis. *A priori* one asks what is the use of any conversion of proteids to albumoses and peptone, if before absorption they are reconverted back to serum-albumin? But the actual experiments are inconclusive. Serum-albumin was not chemically demonstrated to arise from the "peptone" introduced into the intestine. The fact that a solution of "peptone" removed after a 15 minutes' sojourn in a loop of gut in a dog with a Vella fistula, and subsequently introduced into a frog heart manometer, is nutrient to the heart, while previously it was not, is no proof that the nutrient substance has been manu-

factured by the gut epithelium out of the introduced "peptone." Such nutrient material (probably serum-albumin) could arise from the secretion of the gut wall, and Brinck's statement that because pancreatic "peptone" (apparently not purified in any way) after residence in the gut does not sustain the heart, that therefore the notion of addition of proteids from the gut wall is excluded, is easily met by the statement that pancreatic peptone unless carefully treated contains many bodies that are probably so poisonous to the heart that the nutritive effect of any added serum-albumin would be annulled[1].

I concluded therefore that trichloracetic acid suited my purpose as a precipitant of all added proteid material.

The next point was to get a commercial "peptone" which suffered as little loss as possible on treatment with hot trichloracetic acid solution. Witte's "peptone" gives a very heavy albumose precipitate in the cold with an equal volume of 10 % trichloracetic acid, and this precipitate is not entirely cleared up by heating to 100° C., on the other hand Grübler's "peptone" precipitated by alcohol[2] gives only a slight precipitate, and this begins to clear at about 65° C. and is dissolved at 70° C.

I therefore compared the loss of nitrogen on samples of these products by treatment of their solutions with trichloracetic acid at a temperature of 80° C.

Grübler's "peptone" by alcohol.

1·3408 gram (water and ash free) by Kjeldahl method neutralised 31·07 c.c. $\frac{N}{2}$ sulphuric acid, *i.e.* 16·22 % N.

1·3602 gram dissolved in water and equal volume of 10 % trichloracetic acid added. Heated to 80° C. and filtered hot (hot water funnel at 80° C.).

Filter and beaker thoroughly washed with hot 10 % trichloracetic acid and filtrate and washings evaporated to dryness and taken up in strong sulphuric acid. On distillation 31·48 c.c. $\frac{N}{2}$ sulphuric acid neutralised = 16·20 % N,

i.e. 99·88 % of the N recovered.

[1] See also the criticism of these experiments by R. Neumeister. *Zeitschr. f. Biologie*, XXVII. 338. 1890.

[2] This "peptone" is prepared by the method of Henninger and is remarkable for its small ash. Henninger quotes the ash at ·31 % for fibrin-peptone and ·54 % for albumin-peptone. The sample I used held ·51 % of ash, while that of a sample of Witte's peptone was 2·8 %.

Witte's "peptone."

·9138 gram (water and ash free) by Kjeldahl method neutralised 21·57 $\frac{N}{2}$ sulphuric acid = 16·52 % N.

1·442 gram dissolved in water and equal volume of 10 % trichloracetic acid added. Heated to 80° C. and filtered hot (hot water funnel at 80° C.). Filter and beaker washed with hot 10 % trichloracetic acid and filtrate and washings evaporated to dryness and taken up in strong sulphuric acid. On distillation 32·28 $\frac{N}{2}$ sulphuric acid neutralised = 15·67 % N,

i.e. 94·86 % of the N recovered.

Probably by boiling the Witte's "peptone" and trichloracetic acid mixture instead of only heating to 80° C. the loss would have been far less, but the experiment was advisedly made at 80° C. since in separations of native albumins from albumoses and peptone by this method, there is danger at 100° C. of production of albumose.

Obviously then on this count Grübler's alcohol precipitated "peptone" is preferable to that of Witte, since at a temperature of 80° C. one can operate with trichloracetic acid without loss.

That there is no production of albumose as a result of heating serum-albumin together with Grübler's "peptone" in solution with trichloracetic acid at 80° C. is shown by the following experiment.

1·1453 gram (water and ash free), Grübler's "peptone," dissolved in water and ·5 gram serum-albumin added. Equal volume of 10 % trichloracetic acid added and heated to 80° C. Filtered at 80° C. and serum-albumin precipitate washed by suspension in 10 % trichloracetic acid at 80° C. Washings added to original filtrate and evaporated to dryness, taken up in strong sulphuric acid, and nitrogen estimated. 26·33 c.c. $\frac{N}{2}$ sulphuric acid neutralised = 16·09 % N.

The recovery of the nitrogen of the Grübler's "peptone" is not quite so good here as in the previous experiment, a fact attributable to the difficulty of thoroughly washing the heavy serum-albumin precipitate. In the physiological experiments however the ratio of the succus proteids to the "peptone" is of course far lower than in this experiment.

As a result of these preliminary experiments it was decided to use Grübler's "peptone" as test proteid, and to effect its separation

from the proteids of the succus by trichloracetic acid at 80° C. added to 5 % of the mixture removed from the gut.

(*b*) *The quantitative estimation of the "peptone."*

Peptone has been estimated by Colorimetric methods based upon the biuret reaction, by a Polarimetric method based upon the specific rotation, and more recently by means of a Nitrogen factor.

The colorimetric method was introduced by Schmidt-Mülheim, and simply consists in making a standard tint by colouring a peptone solution of known percentage with copper sulphate and sodium hydrate, and either diluting the solution to be estimated, after similar treatment, to the tint of the standard, or *vice versâ* if less saturated than the standard. To work this method one must have either pure peptone or albumose, or a mixture in which the ratio of one to the other is constant, and furthermore extraneous colouring matter (bile pigment) must be absent. If one uses a mixture of albumoses and peptone as test proteid, there is no guarantee that the ratio in the fluid removed from the gut is the same as that in the solution of known strength introduced, for Kühne, and Chittenden and Amerman have shown that the albumoses are not so diffusible as peptone; thus Kühne gives the diffusibility of albumoses and peptone through vegetable parchment into water as, Deuteroalbumose : Protoalbumose : Antipeptone :: 10 : 19 : 51, and the antipeptone here was not absolutely free of albumoses. The colour of the biuret reaction with albumoses is far bluer than that with peptone, and hence the original standard tint under the conditions of experiment is no basis of comparison for that obtained in the fluid removed from the gut.

Bile staining of the fluid removed from the gut is also a possibility and animal-charcoal decolorisation leads to loss of peptone (Schmidt-Mülheim).

Klug has used the spectro-photometer in conjunction with the biuret reaction; a refinement of the ordinary process. A reference to his figures shows that the light absorption-ratio for Kühne's peptone is 2·65 to 2·635, while Hemialbumose is stated at ·944 to ·91. Since the concentration of a "peptone" solution is calculated from the product of its extinction coefficient by the absorption-ratio, the latter must naturally be the same in the solution of known percentage and that to be determined, but again will not be so necessarily in the case under consideration on account of the greater diffusibility of the peptone in the mixture and the great difference in the light absorption-ratio for albumose and peptone.

The polarimetric method has been used by Hofmeister after separation of other proteids by boiling with ferric chloride and sodic acetate. It cannot be claimed for the polarimeter that it is an accurate instrument for quantitative work even in the case of the estimation of sugars whose specific rotations are fairly definite. To what extent it can be of value with proteid solutions can only be imagined from a glance at the appalling differences in the values given for the different proteids and for one and the same proteid prepared by different methods, to be seen in the text-books. The method has however been depended upon by v. Wittich and Röhmann in work upon intestinal absorption.

The nitrogen factor method in everyday use in commercial analysis of foodstuffs, and recently used by Sebelien and Immanuel Munk for estimating the proteids of milk, has so far as I am aware only been employed by Brandl for the estimation of "peptone," in his experiments upon absorption from the stomach. I consider the method specially suitable for the work under consideration, because the analyses of albumoses and peptone have not shown that there is any marked difference in the nitrogen content of the two classes of proteid, and consequently change in the relative proportions of one to the other in a mixture does not seriously affect the estimation.

Chittenden and Goodwin quote Myosin-peptone at 16·2, 16·7, 16·68 and 16·9 % N, Protomyosinose at 16·92 %, Deuteromyosinose at 17·00 %. Kühne gives for Antipeptone from tryptic digestion of Witte's "peptone" 16·4 % N. Again Dr Gamgee on pp. 132 and 143 of Vol. II. of his *Physiological Chemistry of the Animal Body* gives two tables with nine estimations in each by Kühne and Chittenden of the percentage of nitrogen in albumoses and peptones (though the peptones in these tables are not as free of albumoses as by Kühne's most recent methods) in which the mean for the albumoses is 17·14 % and for the peptone 17·16 %.

It must be admitted however that a consideration of the differences in nitrogen percentage of peptone prepared from different sources and by different methods makes one inclined to think with Pekelharing that peptone can at present hardly be considered as a chemical entity.

In devising a process of estimation upon this basis the following points have needed consideration, (*a*) Freeing the sample of "peptone" used as far as possible from nitrogenous matters other than albumoses and peptone. (*b*) The satisfactory precipitation of the "peptone" in order to collect it for estimation of nitrogen. (*c*) The washing of the

precipitate to remove any adherent nitrogenous matter, originating in the succus entericus, other than that of proteid nature previously removed by the treatment with trichloracetic acid.

The principal nitrogenous bodies likely to contaminate a commercial "peptone" are leucine, tyrosine, aspartic and glutamic acids, lysine and lysatine. The richly nitrogenous xanthine bodies, if present, will only occur in traces, since according to Kossel they arise not from the fibrin originally submitted to digestion, but from the nuclein of nuclei of leucocytes entangled in the meshes of the fibrin during coagulation of the blood.

According to Lea 8 to 10 % of leucine and 2 to 3 % of tyrosine are formed from proteid during tryptic digestion, and the leucine is considerably richer in nitrogen than the tyrosine (10·69 to 7·73 %).

Hedin obtained only 7·25 grms. of lysine (19·18 % N) reckoned on the platinum salt from the pancreatic digestion of 3000 grammes of moist fibrin, say ·242 % of the moist fibrin.

I have no data as to the amount of aspartic and glutamic acids split off from proteid during pancreatic digestion.

According to Hirschler the proportion of nitrogenous bodies other than albumoses and peptone present in a commercial sample of "peptone" can be gauged by estimating the nitrogen of the filtrate after treating the sample with phosphotungstic acid in the presence of a mineral acid (sulphuric) and reckoning it in per cent. of the total nitrogen of the sample. This statement is made on the assumption that peptone is completely precipitated by phosphotungstic acid so that none appears in the filtrate. This is not the fact according to both Neumeister, and Kühne and Chittenden; also my own experience agrees with that of these observers.

Having a small quantity of peptone prepared by Kühne's method of salting out the albumoses, with ammonium sulphate saturation, in neutral, acid, and alkaline solution, with subsequent removal of the ammonium sulphate by barium carbonate, and final precipitation of the peptone by absolute alcohol, I put this point to the test. ·2370 gramme of "Kühne's peptone" were dissolved in water and sulphuric acid (previously diluted) added to 10 % of the solution and saturated solution of phosphotungstic acid till precipitation was over. The filtrate was evaporated down and taken up in 20 c.c. of strong sulphuric acid and the nitrogen estimated by the Kjeldahl method. The filtrate neutralised ·59 c.c. $\frac{N}{2}$ sulphuric acid = 1·74 % nitrogen, so that taking

Kühne's peptone at 16·4 % of N, 10·54 % of the total nitrogen appeared in the filtrate.

Now in this sample the mass of the leucine and tyrosine must have crystallised out from the hot ammonium sulphate solutions of peptone on cooling, and the subsequent alcohol treatment must have aided in the further removal of leucine, so that it is impossible to escape the conclusion that the bulk of the nitrogen in the filtrate originated from unprecipitated peptone. Pure peptone is the chimæra of the physiological chemist, and Kühne himself says, "Experience however has yet to prove whether in reality it is possible to obtain Antipeptone perfectly free from amido-acids, for I have observed that nearly dry preparations, which we considered as pure in this respect, after some years' keeping presented here and there tyrosin crystals recognisable by the microscope."

The following nitrogenous bodies are not precipitated by phosphotungstic acid, and if present would appear in the filtrate: leucine, tyrosine, aspartic and glutamic acids, kreatin, glycine and urea. On the other hand lysine (Drechsel and Siegfried), kreatinine (Körner) and xanthin bodies (Hofmeister) are precipitated by phosphotungstic acid.

I extracted the Grübler's alcohol-precipitated "peptone" for eight hours (in a Drechsel's extractor) with hot alcohol, to remove any leucine still present. Lysine is slightly soluble in hot alcohol but tyrosine, aspartic and glutamic acids are not. The fact however that this type of commercial "peptone" is prepared by peptic digestion and not by tryptic lessens the danger of the presence of such bodies to any great extent.

The following are four analyses of the material used after alcohol extraction.

·4327 gram (ash and water free) neutralised 10·03 c.c. $\frac{N}{2}$ sulphuric acid = 16·22 % N.

·6120 " " " " 14·14 c.c. $\frac{N}{2}$ sulphuric acid = 16·17 % N.

·8270 " " " " 19·12 c.c. $\frac{N}{2}$ sulphuric acid = 16·18 % N.

·6042 " " " " 14·02 c.c. $\frac{N}{2}$ sulphuric acid = 16·23 % N.

Mean of the four 16·20 % N.

A large stock of this "peptone" was therefore laid in for work.

The next point to be considered was a satisfactory method of precipitation of the "peptone" for purposes of its introduction into the Kjeldahl flask for estimation of nitrogen. The "peptone" after removal from the gut and precipitation of the albuminous bodies of the succus entericus will be in solution in water with 5 % of trichloracetic acid. The first attempts were with alcohol. The solution was reduced to small bulk, neutralised, and absolute alcohol added. I was surprised at the incompleteness of the precipitation by 10 volumes and even by 20 volumes of absolute alcohol, but find that Hofmeister, Neumeister, and Otto have already noted the fact. Otto states that the purer the "peptone" (by which I imagine he means the freer it is from albumoses) the more soluble it is in alcohol. He recommends slight acidification with sulphuric acid and constant shaking, a method I adopted.

Precipitation of Grübler's "peptone" by alcohol.

·5560 gram (water and ash free) treated with trichloracetic acid to 5 % of the solution, filtered, evaporated to small bulk on water bath and neutralised.

10 volumes of absolute alcohol (kept over dehydrated copper sulphate) added. Mixture rendered faintly acid with sulphuric acid and left on a shaking apparatus over night.

Nitrogen of precipitate and filtrate taken by Kjeldahl method.

Precipitate neutralised 4·56 c.c. $\frac{N}{2}$ sulphuric acid = 5·74 % N.

Alcoholic filtrate „ 7·78 „ „ = 9·79 % N.

Hence taking Grübler's "peptone" at 16·2 % N, only 35·43 % of the total nitrogen was in the precipitate, while 60·43 % was in the filtrate and 4·14 % was lost.

Again ·8832 gram (ash and water free) of the "peptone," after receiving the above treatment, was treated with 20 volumes of absolute alcohol with faint acidification and shaking as above.

Precipitate neutralised 10·37 c.c. $\frac{N}{2}$ sulphuric acid = 8·22 % N.

Alcoholic filtrate „ 9·45 c.c. „ = 7·49 % N.

So that 50·74 % of the total nitrogen was in the precipitate, 46·26 % in the filtrate and 3 % lost.

I next used phosphotungstic acid, for though not a complete precipitant, it is I believe the best we have, and for the purpose of determining the working factor of the "peptone" used under the experimental conditions, the main point after all is constancy in the percentage of the original amount precipitated, though of course the

error is diminished the nearer the precipitation approaches completeness[1]. The precipitate with this reagent on heating and subsequent cooling takes on an almost mineral character and is easily handled and worked. The solubility of this precipitate was first determined in order to decide the method of washing. The importance of washing is due to the fact that there may be nitrogenous substances other than albumoses in the succus entericus, and these must be removed. Urea we know from Pregl's analyses is present in the succus. This will not be precipitated by phosphotungstic acid, but some may adhere to the precipitate and precautions must be taken to remove it. So far as I am aware this is the only nitrogenous crystalline body yet detected in succus entericus, but thoughts of kreatinine, which is precipitated by phosphotungstic acid, have made me careful to see that if this body be present it is removed before the final estimation of nitrogen upon which the "peptone" residue is calculated. I prepared a considerable amount of this precipitate, precipitating a solution of Grübler's "peptone" (extracted with alcohol), acidified by addition of $\frac{1}{10}$ of its volume of hydrochloric acid, with phosphotungstic acid in saturated solution. This was washed in 10 % phosphotungstic acid solution, dried, powdered, and dried to constant weight at 110° C.

Solubility of nitrogenous portion of phosphotungstic acid precipitate of Grübler's "peptone."

	Per cent. of nitrogen	Loss of nitrogen % on washing
Precipitate only	4·75 (mean of 3 determ[s])	
Washed *boiling absolute alcohol*	4·70	1·05
Washed boiling 10 % trichloracetic acid holding 10 % phosphotungstic acid	4·49	5·47
Washed boiling 10 % phosphotungstic acid holding 10 % sulphuric acid	4·48	5·68
Washed boiling water	4·35	8·42

The only safe washing therefore is by absolute alcohol, and since urea and the phosphotungstic acid precipitate of kreatinine are soluble in this, I adopted it.

It was next necessary to determine whether by long action upon the "peptone" the trichloracetic acid in any way affected its precipitation by phosphotungstic acid. I determined the percentage of

[1] Sebelien demonstrates tannic acid to be a very imperfect precipitant for albumoses and peptone, though excellent for native albumins.

the total nitrogen of the "peptone" precipitated by treatment of its aqueous solution by phosphotungstic acid and hydrochloric acid, and then compared it with that of some of the same "peptone" heated for some time to 80° C. with 5 % of trichloracetic acid in the solution.

·3327 gram "peptone" precipitate by phosphotungstic and hydrochloric (10 %) neutralised 7·00 c.c. $\frac{N}{2}$ sulphuric acid = 14·72 % N = 90·86 % of the nitrogen of the "peptone" in the precipitate.

·3083 gram "peptone" heated in solution with 5 % of trichloracetic acid for 10 minutes at 80° C., then precipitated by phosphotungstic and hydrochloric acids neutralised 6·40 c.c. $\frac{N}{2}$ sulphuric acid = 14·53 % N = 89·56 % of the nitrogen of the "peptone" in the precipitate.

·3348 gram "peptone" heated in solution with 5 % trichloracetic acid for 20 minutes at 80° C., precipitated by phosphotungstic and hydrochloric acids neutralised 6·83 c.c. $\frac{N}{2}$ sulphuric acid = 14·28 % N = 88·14 % of the nitrogen of the "peptone" recovered.

It is evident therefore that the precipitability is lessened by increase of time of action of the trichloracetic acid on the "peptone." If therefore the yield of precipitated "peptone" is to be as large as possible this action must be kept as slight as possible.

It is of no value to neutralise with sodic hydrate and then evaporate to small bulk before precipitation with phosphotungstic acid because the sodic carbonate developed from the sodic trichloracetate by heating appears also to affect the precipitability in the end, thus

·5238 gram of "peptone" was heated to 80° C. in solution with 5 % of trichloracetic acid for 10 minutes, filtered and at once neutralised with sodic hydrate. The mixture was then evaporated to small bulk (occupying a period of half-an-hour), during which process it had become strongly alkaline from the sodic carbonate (developed from the sodic trichloracetate). It was again neutralised with trichloracetic acid after cooling and precipitated with phosphotungstic and hydrochloric acids.

9·95 c.c. $\frac{N}{2}$ sulphuric acid were neutralised = 13·3 % N = 82·1 % of the total nitrogen precipitated.

Yet immediate neutralisation and precipitation without reduction in bulk on the water bath (theoretically the best procedure), is inconvenient in practice on account of the bulk of the solution to be handled.

In the end I sacrificed the maximum yield of "peptone" precipitate

by the method, to the convenience of evaporating down the trichlor-acetic solution, and as will be seen from the results, the recovery and factor are fairly constant when the quantity of trichlor-acetic acid used and the time occupied in the reduction on the water bath are kept as constant as possible.

The following was the procedure adopted:

To the peptone solution is added an equal volume of 10 % trichloracetic acid solution, the mixture heated to 80° C., filtered through a Schleicher and Schüll paper, No. 589, in a hot water funnel at 80° C. into a porcelain evaporating dish. The filter and beaker are washed into the dish with 10 % trichloracetic acid solution kept hot.

The contents of the dish are now evaporated down to about 50 c.c. and 5 c.c. hydrochloric acid added and phosphotungstic acid in saturated solution drop by drop from a pipette with stirring till there is no more precipitate on adding the next drop.

The fluid is allowed to cool since hot phosphotungstic solution is a better solvent of the phosphotungstic precipitate of "peptone" than when cold. The precipitate is broken up fine with a glass rod and washed on to a Schleicher and Schüll paper, No. 597, in a funnel attached to a filter-pump, a platinum cone being used. This washing is effected by 10 % phosphotungstic acid holding 5 % of sulphuric acid. After the filtrate has once more been tested with phosphotungstic solution to see that there is no more precipitate, the filter paper and precipitate are dried in a steam oven at 90° C.

When dry the paper and precipitate are introduced into a long-necked Kjeldahl flask, about 50 c.c. absolute alcohol added, and the whole briskly boiled for a few minutes. The alcohol is decanted through a small filter paper, which is finally also added to the contents of the flask. The ordinary Kjeldahl nitrogen estimation follows.

Two points in this procedure call for remark. The first is that the phosphotungstic acid should not be added beyond what is required to cause complete precipitation because the precipitate becomes soluble in excess.

I quote an experiment in illustration.

·793 gram (water and ash free) of Grübler's "peptone" after solution in 20 c.c. of water holding 2 c.c. hydrochloric acid was precipitated by an *equal volume of saturated phosphotungstic acid solution.*

6·91 c.c. $\frac{N}{2}$ sulphuric acid were neutralised = 6·1 % N.

Taking Grübler's "peptone" at 16·2 % N this means that 62·35 % of the nitrogen remained in the solution.

The other point is the absolute necessity of driving off the water from the phosphotungstic precipitate before boiling it in alcohol. Just as a very slight addition of water to absolute alcohol makes it a fair solvent of "peptone," so the phosphotungstic compound becomes more and more soluble in alcohol as the percentage of water increases.

I quote an experiment in illustration of this point.

·8845 gram Grübler's "peptone" dissolved in 30 c.c. water and 30 c.c. of 10 % trichloracetic acid added; filtered, reduced in volume on water bath and hydrochloric acid added to 10 %, and saturated phosphotungstic acid solution to complete precipitation.

Filtered and precipitate and paper at once transferred to Kjeldahl flask *without drying*. Alcohol added and boiled. Alcoholic filtrate kept. Nitrogen of precipitate and alcoholic filtrate (after evaporation to dryness) estimated.

Precipitate neutralised 11·95 c.c. $\frac{N}{2}$ sulphuric acid = 9·45 % N.

Alcoholic filtrate 4·12 c.c. $\frac{N}{2}$ „ „ = 3·26 % N.

So that 58·33 % of the total nitrogen was in the alcohol-washed phosphotungstic precipitate, 20·12 % in the alcoholic filtrate and 21·55 % remained in the original phosphotungstic filtrate.

I proceeded therefore to determine the factor for the Grübler's "peptone" (extracted with alcohol) on a process exactly similar to that which the "peptone" removed from the gut in the experiments would have to undergo.

Determination of factor for Grübler's "peptone" precipitated from solution in 5 % trichloracetic acid by phosphotungstic and hydrochloric acids.

Weight of peptone (ash and water free) in grammes	No. of c.c. of $\frac{N}{2}$ sulphuric acid neutralised	% of N	Factor
·7301	13·32	12·77	7·83
·7369	13·62	12·93	7·73
·7981	14·89	13·06	7·65
·8879	15·93	12·56	7·96
·8943	16·22	12·70	7·87
·5730	10·63	13·00	7·69
·6625	11·94	12·61	7·93

Mean factor on the seven determinations 7·81.

It appears from the above that a little less than 80 % of the nitrogen of the original peptone is recovered when it is precipitated from solution in trichloracetic acid after the procedure above described.

The modification of the Kjeldahl nitrogen method employed was that in use in the chemical department of our college, and my thanks are due to the staff of that department for much kind advice during the conduct of this work.

In the preliminary oxidation in sulphuric acid a globule of mercury was introduced into the flask and eight grammes of anhydrous potassium sulphate for each 20 c.c. of strong sulphuric acid, to raise the boiling point. The heating was continued for from 4 to 5 hours, and potassium permanganate was introduced (after cooling) towards the end of the process. The contents of the flask were treated before distillation with 100 c.c. of 40 % sodic hydrate solution holding 1 % of sodic sulphide, for each 20 c.c. of strong sulphuric acid originally used, and "bumping" was prevented during distillation by capillary glass tubes sealed at one end and introduced into the flask open end downward. The distillation flask was fitted with a froth trap, and the flask holding the standard acid (of carefully determined factor) was provided with a trap of glass beads, through which the acid was introduced, and which was afterwards tinted red with methyl orange, so that an escape of ammonia would at once tell its tale by the change of colour in the bead trap. Jena glass was used throughout and fracture of flasks thus reduced to a minimum. Titrations, in which methyl orange was indicator, were repeated twice, or if not concordant three times, a burette of standard acid being in use besides that holding the standard alkali. The nitrogen of the reagents was determined with each change of sample and allowed for in the calculations.

Any excess of phosphotungstic acid in the peptone precipitate does not influence the result as regards nitrogen percentage, a fact already noted by Hirschler, but of which I quote an instance.

·5405 gram Grübler's "peptone" introduced into flask with 5 grams of solid phosphotungstic acid and 30 c.c. strong sulphuric acid and the heating conducted in the ordinary fashion, "bumping" being reduced by a piece of unglazed porcelain.

On distillation 12·48 c.c. $\frac{N}{2}$ sulphuric acid were neutralised = 16·16 % N.

Nevertheless I have always dissolved off the tungstic acid precipitate that forms on the walls of the oxidation flask, in the sodic hydrate used

for distillation, thinking it possible that some traces of ammonium sulphate might be mechanically retained in this precipitate.

Since there are many phosphotungstic acids I bought in a large stock from Kahlbaum as the precipitation may be affected by differences in sample. The volumetric apparatus was by Kaehler and Martini and guaranteed by the "Normal-Aichungs-Commission" of Berlin.

(*c*) *The recovery of the unabsorbed "peptone" from the gut.*

The intestine by virtue of the peculiarities of its mucous membrane (villi and valvulæ conniventes) is a difficult tube to wash, so much so that I cannot understand how reliance can be placed upon washings of loops during life, such as occurs in absorption experiments with a Vella fistula. As will be seen from the second part of this paper, I have only performed one experiment with each animal, in order that I might be able to remove the gut for thorough washing outside the body.

The method of recovery of the unabsorbed solution that I have adopted is as follows. The contents are emptied out into a measuring vessel, the draining being followed by stroking the gut between finger and thumb. The loop is then distended with cold distilled water, clamped at both ends, kneaded well, and the first washing collected in a flask; this is repeated with five successive quantities of water, the washings being added together. Cold water is used advisedly, to cause contraction of the villi and so facilitate the washing of their interspaces, to coagulate the epithelial cells and hinder removal of absorbed "peptone" from them.

The washings are then boiled, filtered, and finally boiled down to about 30 c.c. and added to the original drainings for estimation. About 250 c.c. of wash water are used for a 30 cm. loop of gut, or about 400 c.c. for a 50 cm. loop.

I append the results of two experiments in washing loops of dead gut to show that this method is satisfactory.

Recovery of peptone from dead gut.

(*a*) 50 cms. well washed ileum of dog 24 hrs. dead.
Introduced 50 c.c. of 1 % Grübler's "peptone." Left in 3 minutes.
Recovered directly 48 c.c. of fluid.
Washed gut with five successive lots of 80 c.c. of distilled water.
Washings boiled down and added to fluid directly recovered.

Put through trichloracetic and phosphotungstic process and N estimated by Kjeldahl method.

9·04 c.c. $\frac{N}{2}$ sulphuric acid neutralised = ·06328 gram N.

Taking factor for "peptone" at 7·81 = ·4942168 gram "peptone,"
"Peptone" lost = ·0057832 gram,
i.e. 98·84 % of original "peptone" recovered.

(*b*) 30 cms. well washed jejunum of dog 1 hr. dead.
Introduced 30 c.c. of 2 % Grübler's "peptone." Left in 2 minutes.
Recovered directly 32 c.c. of fluid.
Washed gut with five successive lots of 40 c.c. of distilled water.
Washings boiled down and added to fluid directly recovered.
Trichloracetic and phosphotungstic method, and estimation of N by Kjeldahl method.

10·8 c.c. $\frac{N}{2}$ sulphuric acid neutralised = ·0756 gram N.

Taking factor for "peptone" at 7·81 = ·590436 gram "peptone,"
"Peptone" lost = ·009564 gram,
i.e. 98·41 % of original "peptone" recovered.

Section II. Physiological.

In the conduction upon an animal of experiments upon absorption by the gut, one may use loops of gut exposed immediately before use or a fistulous loop isolated after the manner of Vella. The advantage claimed for the Vella loop is that it is in a more normal condition than is a loop directly exposed and fitted with cannulæ. But on the other hand a Vella loop is a piece of gut that has been functionless for many days, and is moreover extremely liable to catarrh, it is difficult to fill, more difficult to wash, and useless for purposes of excitation of mesenteric nerves. To these disadvantages may be added that of the actual experiment and its control having to follow one another (unless 2 Vella loops be isolated, an operation I believe not yet successful), so that the condition of the gut may be different during two experiments which should be strictly comparable. Of recent years the Vella loop has been used by Gumilewsky and Röhmann, the recently exposed loop by Lannois and Lépine, Leubuscher, Heidenhain, Edkins, and Tecklenburg.

I have used the method with recently exposed loops, which, when one has acquired the necessary skill, is I believe more reliable than

that with a Vella loop and allows of experimental work impossible in the latter case. Leubuscher introduced the method of using a control loop from the same region of the intestine, and certainly if the difference between the absorptions of the two loops, under exactly similar conditions, is small, the method is preferable to successive experiments upon one loop, since one cannot guarantee that the manipulation necessary for the first emptying of the loop shall not so interfere with its physiological condition, that the second absorption does not occur under conditions strictly comparable with the first.

The great difficulty in the two loop method lies in the measurement of the loops. A measurement of a piece of gut in a living animal must be taken once and for all, for such a muscular tube may under untoward conditions elongate or contract to the extent of a quarter of its normal length, by which I mean the length as measured in an animal with spontaneous peristalsis reduced by morphia, and immediately after opening the abdominal cavity under warm cloths. The whole procedure of measurement is a matter of experience, and the skilled operator measures two loops during life whose *post mortem* length does not differ by more than a centimetre. The method I have adopted is to measure with a piece of waxed thread inch by inch, and to at once mark on the surface of the length of gut selected the points for subsequent insertion of cannulæ, with a glycerine and methyl violet ink.

Dogs have been used as experimental animals, receiving 3 to 5 milligrammes of morphia per kilo body weight, and being subsequently anæsthetised with chloroform. The procedure in the experiments was as follows: the dog having fasted 24 hrs. was anæsthetised with morphia and chloroform, tracheotomised, and the abdomen opened in the *linea alba*. The cæcum was picked up and a measurement of 100 to 150 centimetres (according to the size of the dog) taken up the gut to the point of commencement of the lower loop. A mark was made with the ink and two loops carefully taped off of the same length, their origins and terminations being marked with the ink. Only a knuckle of the gut need be outside the belly at any moment during this process.

The gut was then opened at the marks for each loop, tapeworms, if present removed, and cannulæ with rubber tubes and clips tied in. A stream of 2 litres of normal saline at 40° C. and at a pressure of 20 cms. of water was then passed through each loop, the direction being changed from time to time to facilitate the removal of "flocks" of adherent material.

After the termination of the washing, a period of fifteen minutes was allowed to elapse, to permit the absorption of any residual saline, in order that the peptone solution might be introduced at approximately the concentration at which it was prepared.

The gut was now brought out of the abdominal cavity, the two loops lying on either side between flannels wrung out in normal saline at 42° C. The actual absorption experiment was conducted outside the belly because the earlier experiments showed that it was not possible to obtain anything like equality of absorption in the two loops, when they lay inside the belly, simply, I imagined, as a result of kinking of the mesentery and interference with the blood supply.

This method has been used by Edkins, and since the experiment only lasts 15′ and it is very easy to keep up warmth and moisture by warm flannels wrung out in saline, the deviation from a normal condition is not great, and one has the great advantage of being able at any moment to lift the cloth and see the gut and mesentery.

The "peptone" solution was now run in to the two loops simultaneously, the moment of opening the burette clips being noted as the commencement of the experiment. Two separate vessels, jacketed with hot water, held the "peptone" solution, in which a thermometer and gas regulator bulb were immersed. The solution was kept at 41° C. and just before use was sucked into burettes attached to the stock vessels, and run from the burettes into the gut loops.

It is convenient to faintly tint the peptone solution and to attach small vertical tubes to the cannulæ in the lower ends of the loops, so that should the peptone solution tend to run quickly through the loop, its appearance in the small vertical tube is at once evident, and it can be blown back and the clip closed. Rose Bengal, a non-nitrogenous and harmless dye stuff, is convenient for the purpose.

The quantity of solution introduced was regulated by the length of loop so that distension was avoided; as a rule as many cubic centimetres of solution were introduced as there were centimetres of loop. After the experimental time had elapsed all but one minute, threads were placed through the mesenteries of each loop, and at the moment of cessation of experimental time were tied so as to completely stop the circulation. The medulla of the dog was destroyed, the loops excised and treated in the manner already described.

To demonstrate how well the method works, I append a table showing the results of six experiments.

TABLE I.

I	II	III	IV	V	VI	Loop	VII	VIII	IX	X	XI
Number of Exp.	Weight of dog in kilo-grammes	Distance of lower loop (in centimetres) from ileocæcal valve	Length of loops in centi-metres	Duration of exp. in minutes	Quantity of "peptone" introduced in grammes		Grammes of nitrogen recovered	Grammes of "peptone" recovered	Grammes of "peptone" absorbed	Per cent. of "peptone" introduced that was absorbed	Difference between the two loops
I	18·5	100	40	15	1·00	Upper	·07611	·59442	·40558	40·55	·80
						Lower	·07714	·60246	·39754	39·75	
II	16·0	100	30	15	·72	Upper	·03304	·25804	·46196	64·16	3·62
						Lower	·03639	·28410	·43590	60·54	
III	18·0	150	30	15	·60	Upper	·02240	·17494	·42506	70·84	2·45
						Lower	·02044	·15963	·44037	73·39	
IV	11·5	100	30	15	·60	Upper	·03570	·27882	·32118	53·53	2·74
						Lower	·03780	·29522	·30478	50·79	
V	11·0	100	30	15	·60	Upper	·03304	·25804	·34196	56·99	2·00
						Lower	·03150	·24601	·35399	58·99	
VI	9·5	100	30	15	·60	Upper	·02450	·19134	·40866	68·10	·72
						Lower	·02506	·19572	·40428	67·38	

In all the above experiments the concentration of the "peptone" solution was 2%, the solvent distilled water.
Nitrogen factor for "peptone" 7·81.

It will be seen from this table that the difference between the absorptions in the two loops, taken at a distance of from 100 to 150 cms. from the ileocæcal valve, is less than 5 % on absorption of from 40 % to 70 % of the "peptone" introduced.

In six experiments conducted with the loops replaced in the abdominal cavity the differences between the absorptions of "peptone" in the two loops were 8·97, 19·02, 12·98, 13·92, 7·65, and 12·66 per cent. respectively, and in a case of two successive absorptions in one loop 11·7 %.

It is evident therefore that by the two loop method any difference between the control and experimental loops exceeding 5 % may be considered as a difference induced by the experimental conditions.

February 5th, 1896.

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THE TIME OF CONTRACTION OF THE PAPILLARY MUSCLES.

By JOHN BERRY HAYCRAFT, M.D., and D. R. PATERSON, M.D. (Two Figures in Text.)

(*From the Physiological Laboratory of the University College, Cardiff.*)

Attention has already been directed to the papillary muscles by a very interesting series of papers by Professors Roy and Adami in the *Practitioner* for 1890. In sections I. and II. pp. 82—94, these observers describe an instrument—a myocardiograph—which they invented for the purpose of recording simultaneously both the contraction of the wall of the ventricle and the contraction of the papillary muscle. For full particulars of this instrument the reader is referred to the original paper, the following is but an imperfect description. A window is made in the anterior chest wall of a dog and the heart exposed. A wire hook is inserted through the wall of the auricle and passed thence into the ventricle, where its end is hooked over one of the flaps of the mitral valve. A thread passes from the hook round a pulley (m) to a writing lever. In order to obtain a tracing of the ventricular wall another hook is fixed into the ventricle from the outside, and from this a thread passes over a pulley (f) to the second writing lever. These pulleys are joined mechanically to a moveable upright, which is attached at one end to the outside of the ventricle, at a point whence the papillary muscle springs. When these hooks are approximated to the base of attachment of the papillary muscle the levers are elevated, and when they move further apart the levers recover, by means of fine indiarubber threads.

Now while the instrument appeared to us capable of recording, by the hook of pulley (f), the contraction of the ventricle, for any approximation could in this case be due solely to the contraction of the ventricle, the case for the papillary muscle seemed not quite so clear. During contraction a shortening of the heart, though to a slight extent, probably occurs, the base of the organ certainly diminishes in girth, and with the thickening of the ventricular wall, the musculi

papillares move towards each other and with the obliteration of the ventricular cavity at the end of the systole become approximated. These factors will necessarily affect the relative distance between the mitral valve around which the hook is passed and the wall of the ventricle to which the upright is attached: so that a separation or approximation of these two points is possible over and above what may be due to a contraction of the papillary muscle itself. Obviously the curve obtained from the lever worked by the pulley (*m*) and the hook attached to the mitral flap, will give the greater or less approximation of the hook to the fixed point on the pericardial surface of the ventricle, but it would be erroneous to assume that the results were alone dependent upon the contraction of the papillary muscle.

Under these circumstances it seemed desirable to repeat the experiments, using another and if possible a simpler method of investigation. The plan we adopted was practically the same as that made use of by Fenwick and Overend[1]. A rabbit was killed by a blow upon the head and the heart rapidly removed from the body. The left ventricle was slit open and pinned upon a wooden table, the pins passing through the wall of the ventricle close to the attachment of the papillary muscle. This was therefore a fixed point. Hooks were rapidly inserted, one into the top of a papillary muscle, the other into the neighbouring ventricular wall. From the hooks silk threads passed vertically upwards to writing levers suspended above by elastic springs.

Roy and Adami by the method used by them came to the conclusion that the papillary muscles begin to contract *after* the commencement of the ventricular contraction, and that they finish contracting before the ventricle begins to relax. Fenwick and Overend found that the period which intervened between the commencement of the contraction of the ventricle and that of the papillary muscle was as little as $\frac{1}{10}$ of a second in a fresh vigorous heart, and they consider it probable that in the living heart the two beat synchronously.

This we found to be the case even in our own experiments, for we were able by a little practice, to obtain a tracing of the heart within a minute and a half from the time the rabbit was killed. In all these cases the heart and papillary muscle commenced contracting together, but after a minute or so they got out of time. When this occurred the papillary muscles, in many cases, contracted after the wall of the ventricle, in others they preceded it. In tracings Fig. 1 (*a*),

[1] *Brit. Med. Journ.* 1891, vol. i. 117.

Fig. 1 (*b*), the upper curve is that of the papillary muscle, the lower one that of the muscular wall. In (*a*) the lever attached to the papillary muscle does not write at the level of the paper. Corresponding points of the two curves are given. As the papillary muscle and the ventricle contract the levers are pulled down. After contraction, the elastic bands pull the levers up again. Measuring from the corresponding points it will be seen that in Fig. 1 (*a*) the two curves descend as a result of the muscular pull upon the levers exactly at the same

FIG. 1 (*a*). Read left to right, speed 4 cm. per second.

FIG. 1 (*b*). Read left to right, speed 4 cm. per second.

time. In trace Fig. 1 (*b*), taken from the same heart as the last, three or four minutes later, it will be seen that the heart is beating slowly and that the papillary muscle begins *before* the wall of the ventricle. The heart is dying and the two events are no longer synchronous.

In Fig. 2 (*a*), which is taken from the heart of another rabbit

it will be seen that although the heart is beating slowly and is not so vigorous as that which traced Fig. 1 (*a*), yet the papillary muscle and

FIG. 2 (*a*). Read left to right, speed 4 cm. per second.

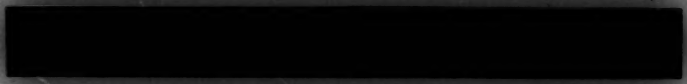

FIG. 2 (*b*). Read left to right, speed 4 cm. per second

the ventricle wall contract together. In Fig. 2 (*b*), a tracing of the same heart a minute or so later, the papillary muscle lags behind the ventricle quite perceptibly.

It is evident therefore that if the observation is made very soon after the death of the animal, and without subjecting the heart to unnecessary manipulation, it is possible to demonstrate what in all probability occurs in the living animal, namely, a contraction of the papillary muscle and the wall of the ventricle occurring synchronously.

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ON THE STRUCTURE AND DEGENERATION OF NON-MEDULLATED NERVE FIBRES.

By IVOR Ll. TUCKETT, B.A., *Fellow of Trinity College, Cambridge.*

(Plates I—IV.)

(*From the Physiological Laboratory, Cambridge.*)

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Introduction.

THE importance of non-medullated nerve fibres lies in the fact that, owing to the absence of medulla, it is possible to examine and measure in the fresh state the structure which carries the nervous impulse. For in the living state alone can one be absolutely certain of the truthfulness of appearances, and if these agree with the appearances obtained after killing a tissue with some fixing reagent, then the latter are probably

true also. If then the structure of non-medullated nerves is once for all definitely determined, the results may very possibly be applied to the axis cylinders of medullated nerve fibres (which in the fresh state are obscured by the medulla), since Kühne and Steiner[1] have shown that the electrical properties of medullated and non-medullated nerves are very similar.

It may, however, be objected that the structure of non-medullated nerves is already perfectly known. To show how far this is from the fact, I proceed to give an historical sketch of the structure of fibres of Remak, especially as there is not, I believe, any such account at present written in English.

Part I. Historical.

In the following account[2] of the views held as to the structure of fibres of Remak, the whole difficulty has centred around the right use and interpretation of the words, *fibre*, *fibril*, and *fibre of Remak*. For when observers have described a sheath, they have not generally made it sufficiently clear to what structure the sheath is supposed to belong. Hence the existence of a sheath of Schwann has been definitely maintained by some authors, but denied by others.

Remak[3(1)], in 1838, first described the grey or organic nerves as consisting of fibres differing from medullated or "primitive" nerve fibres. He described these nerves as transparent and gelatinous in appearance, with their surface nearly always indicating a longitudinal striation and consisting of naked fibres with no sheath. They broke up easily into delicate threads which were accompanied by oval nuclei, by which, as also by their whole appearance, they were easily to be distinguished from connective tissue. These fibres or threads (which constituted the grey nerves, and which have been named fibres of Remak after him) arose in bundles or singly from nerve cells in ganglia.

Remak does not give any definite measurements of the size of these fibres, but from a comparison of the figures he gives, with those

[1] *Untersuchungen des physiologischen Instituts der Universität Heidelberg*, III. 154. 1880.

[2] In this account only the structure of fibres of Remak is considered. The proof of their nervous nature is omitted, as this is an entirely different question. An admirable account of this however will be found in Axel Key and Retzius: *Studien in der Anatomie des Nervensystems und des Bindgewebes*, II. 148. 1876. Stockholm.

[3] The references to literature will be found at the end of this section.

given by other authors, and with the appearances I have myself obtained, the fibres in his figures are about 2μ in diameter[1].

These appearances were criticised by Valentin[4], Rosenthal, Bidder, Volkmann and Kölliker, who all, for various reasons, maintained that they were only another form of connective tissue: Rosenthal in particular asserting that their structure was granular rather than fibrillar, while Valentin especially maintained that they were prolongations of the sheaths of ganglion cells accompanying and surrounding medullated fibres. Muller[5] contested Valentin's view, although he could not convince himself of the origin of fibres of Remak from cells in the sympathetic ganglia.

Remak[6] retorted that post-mortem changes took place in his grey organic fibres to such an extent that they looked very different from the same nerve fibres in the fresh state; and that in this way misapprehensions of his description of these fibres had arisen.

No further very important statements were made about these fibres till Max Schultze[7], in 1863, published a paper on the structure of the olfactory nerve and mucous membrane of various vertebrates. In this paper he described, in the sheep for instance, certain fibres coming from the glomeruli of the olfactory bulb and having a diameter of 50—90μ. They consisted of a distinct nucleated sheath enclosing a granular fibrillar substance which really was constituted by the finest elementary fibrillæ, each being about 3μ in diameter. These fibres he called "primitive" olfactory fibres. Their sheath was rendered transparent by water or acetic acid, whereas weak caustic soda made the sheath more prominent by causing the contents of the sheath to become transparent.

Being struck by the resemblance of the olfactory fibres to Remak's grey organic fibres, he further stated that the two were exactly similar.

As Max Schultze, in particular, is accredited with having discovered a distinct sheath of Schwann for fibres of Remak, it is important to consider the logical outcome of the above statements of his. For from his words it must be concluded, that fibres of Remak are large fibres with a distinct sheath enclosing a mass of fibrils, and Max Schultze[8] himself went so far as to state, in Stricker's handbook, that non-medullated peripheral nerve fibres varied in size according to the number of fibrillæ of which they were composed, so that each fibre was equivalent to an axis cylinder and had a complete nucleated sheath of Schwann; and that the thickness of these

[1] See Pl. 1. Figs. 2, 3, 4 in his paper.

non-medullated fibres varied very greatly, those of the sympathetic hardly exceeding the diameter of a medium medullated fibre while the olfactory fibres of many animals were at least *three* or *four* times the diameter of a large medullated fibre.

Hence Max Schultze definitely states that fibres of Remak have a diameter of 10μ—80μ. But no other author, with the exception of Ranvier, has described fibres of Remak as having a diameter greater than 4μ. Consequently it is obvious that when Max Schultze talks about a distinct sheath for his fibres, he is describing a totally different structure to that described by all other observers as a fibre of Remak. As a matter of fact, the primitive olfactory fibres of Max Schultze have a diameter of 50μ—90μ. And, owing to the similarity of their contents with grey organic nerve fibres, he was led to make the assumption that in the latter also, fibrils were associated together to form a larger primitive fibre, which he supposed must be a fibre of Remak. In reality, the fibrils to which he allowed a diameter of 3μ, and which constituted together his olfactory primitive fibres, alone could agree with the fibres of Remak described by all other authors.

Waldeyer [7] considered the grey fibres of the sympathetic as formed out of bundles of the finest fibrils, corresponding to the fibrillæ of the axis cylinder of a medullated fibre. Each bundle of fibrillæ was surrounded by a delicate nucleated sheath but by no medulla. Saturated solutions of oxalic acid, by dissolving up connective tissue; solutions of caustic potash, by dissolving up the nerve fibres, were sure means of distinguishing fibres of Remak from connective tissue.

Bidder [8] regarded only some of the so-called fibres of Remak as nervous, and these he looked upon as naked axis cylinders.

Next to Max Schultze's account, that given by Axel Key [9] and Retzius is the most classical. I therefore give a resumé of what they say. "On treating a branch of the sympathetic with osmic acid, followed by carmine, a yellowish transparent shining substance is seen longitudinally striated. Throughout this substance are scattered numerous oval, longitudinal, or spindle-shaped nuclei. The limits of the individual fibres of Remak are hardly seen: but they are indicated by longitudinal striations. In fact, the bundles which here and there are distinctly seen to be formed by fibres joined together, can, on teasing, be broken up lengthwise into numerous strips which vary greatly in breadth. These small strips correspond clearly to the so-called fibres of Remak described by many histologists; for they can be isolated as tolerably sharply limited fibres, sometimes cylindrical

but generally more or less distinctly flattened. They appear in the latter case, in profile especially small, in surface view rather wide, or if they are twisted, changing from narrow to wide. The smallest has a breadth of 1·6μ—2·4μ, and the substance of the fibre is homogeneous, transparent, clear to colourless in a fresh condition, but somewhat yellowish-grey after hardening in osmic acid. Here and there may be seen in them, when lying flat, a weak indistinct longitudinal striation. Their broken ends often appear to split up into the finest fibrils, and at times such fibrils appear as a kind of brush-like formation, uniting again, further on, into a fibre. This corresponds to the constitution of a medullated nerve fibre out of fine fibrillæ. But in other cases no such signs of a fibrillar arrangement are found and no longitudinal striation."

They never saw any definite traces of a sheath for a fibre of Remak, except granular protoplasmic formations around and more especially at each end of the nuclei. With regard to the latter: "they are in general small and spindle-shaped, with pointed or somewhat rounded ends. They lie very close to the single fibres of Remak, but may now and then be teased from them partially or completely; in this case no traces of a sheath are seen to cling to them. They vary in length from 12·5μ—20·8μ. They are therefore, on the whole, larger than the nuclei of medullated nerves. At both ends of the nuclei occur generally round yellowish shining granules. Otherwise no peculiar protoplasmic surroundings to these nuclei are to be observed."

Finally, they considered them to agree perfectly with the constitution of olfactory nerve fibres and also to resemble the non-medullated endings of medullated nerves. With silver nitrate they could produce no traces of nodes. The figure[1] which they give of a transverse section of fibres of Remak is very indifferent, as it is impossible to make out any distinct outlines for the individual fibres.

A much better figure, however, is given by Ranvier[(10)] who describes the fibres of Remak as forming little islands which may be resolved into dots of pretty nearly equal diameter, placed side by side. These dots did not correspond to fibres of Remak, but to fibrils, which enter into their composition.

Describing teased preparations, he said the fibres of Remak varied from the thickness of a moderate sized medullated fibre to anything extremely small. They formed a network on the bars of which were oval nuclei. The fibres themselves presented irregular longitudinal

[1] See Fig. 20, Plate xxi. in their work.

striations and could be distinguished from connective tissue by their taking a pink tint with picro-carmine, after fixing in osmic acid. Acetic acid made them swell up and blend together, forming clear ribbons such as Henle had erroneously described as the normal structure of fibres of Remak. The nuclei were always placed on the surface of fibres of Remak.

But his most important observation, relates to the varicose appearance of the fibres after treatment with 2 per cent. ammonium bichromate. Max Schultze, it is true, held that a varicose appearance produced by the action of chromic acid and other reagents, was a sure test of distinguishing peripheral nerves from other structures, but he never made any such definite statement with regard to fibres of Remak as Ranvier's, which is as follows:—"When the nerves have remained several months in a solution of 2 per cent. ammonium bichromate, they have acquired a certain consistency, and it is then easy to dissociate their elements and to colour them with ammonium picro-carminate. In these preparations the fibres of Remak have a characteristic appearance, which again admits of distinguishing them from connective tissue fibres. A well marked varicosity appears in the fibrils of the fibres of Remak, while connective tissue bundles preserve their wavy form and characteristic silky appearance. The fibres of Remak thus consist of a number—more or less considerable—of fibrils placed side by side. Under the influence of ammonium bichromate vacuoles of variable diameter are produced in these diverse fibrils. These vacuoles are so situated as to make the fibrils moniliform, and, as the vacuoles of one fibril closely interlock with the vacuoles of neighbouring fibrils, it is not easy to make out their arrangement to each other in a fibre of Remak without the most careful teasing.

Of the ultimate nature of these fibres, Ranvier is not very clear, for he says: "The finest fibres of Remak may consist of a single nervous fibril; but when the fibres have a considerable diameter, they are composed of several fibrils. In the first place the protoplasm which surrounds their nucleus, is spread over their surface, and forms for them a single sheath: as one observes, for example, on the embryonic nervous tubes, which are enclosed later by myelin, or on the non-medullated fibres into which medullated fibres run at their termination. In the second place, that is to say, when a fibre of Remak is constituted by several nervous fibrils, the protoplasm forms over them a peripheral envelope. And, further, it penetrates between the fibrils in a way to separate and unite them." Of this protoplasmic

sheath, however, Ranvier gives no figure. From this account, then, it is obvious that Ranvier describes as a fibril what Key and Retzius described as a fibre of Remak, but when he says the fibres of Remak vary from the thickness of a moderate sized medullated fibre to anything extremely small, he admits that a single fibril may form a fibre of Remak. Therefore he agrees with Max Schultze in giving a fibre of Remak a diameter of 10μ, but differs from him both in denying the existence of a sheath of Schwann, and in admitting that a fibre of Remak may only have a diameter of 2μ.

Boveri[m] denies the plexiform arrangement of fibres of Remak described by Ranvier, and, like the three previous observers, notices that non-medullated nerves can be broken up into parallel fibres, but he does not consider that bundles of these fibres belong in any way together, as Max Schultze thought, so as to form a higher whole, *i.e.* a nerve-fibre, especially as he agrees with Ranvier in denying a definite sheath of Schwann, such as was described by Max Schultze. But he is not in accord with Ranvier in considering that a fibre of Remak may consist of either a single fibril or of a bundle of fine fibrils; rather he denies Ranvier's statement that the nuclei always lie on the surface of the fibrils. For, describing the nuclei as often embedded in such fibrils, he considers as his ultimate element—or fibre of Remak—a single one of these fibrils which, as we have seen, is the same as the fibre of Remak of Key and Retzius.

"A fibre of Remak," he says, "consists of an axis cylinder, surrounded by a nucleated sheath of Schwann, partly consisting of myelin." Consequently he regards each fibre of Remak in a non-medullated nerve as equivalent to a complete medullated nerve, with the single difference that it is not segmented. He bases this opinion:

(1) Chiefly on his transverse section appearances after treating non-medullated nerves by the Maley-Kupffer method of staining[1]. For here he figures a fibre of Remak as showing a red-coloured dot representing the axis cylinder, lying within a less deeply coloured circle, representing the sheath[2].

(2) He says that osmic acid shows, in transverse section, the axis cylinder fibril of a medullated fibre as a homogeneous point, while a fibre of Remak, on the other hand, merely exhibits a grey coloured

[1] *Zeit. f. wiss. Mik.* 1885. 106.

[2] See Pl. II. Fig. 20 in his paper.

ring with a colourless centre, which becomes a red dot after being treated with acid fuchsin.

(3) He says that all preserving fluids, except osmic acid, make the contents of the axis cylinder space of a medullated fibre congeal into a central solid rod, while a fibre of Remak does not shrink, but preserves its independence after treatment with all preserving fluids.

(4) The axis cylinder fibril of a medullated fibre is accompanied by no nuclei, whereas he considers that the nuclei of a fibre of Remak are embedded in the circular sheath surrounding the red dot shown in his figure, "because" he says, "the nuclei can never be isolated but always cling to the fibres."

(5) The axis cylinder fibril of a medullated nerve is four or five times smaller than a fibre of Remak.

From all these data he concludes that a fibre of Remak is a combination of constituents and not a single structure, like the homogeneous axis cylinder fibril of medullated nerves.

His reasons for considering these fibres to be constituted with a medullated sheath are as follows:

(1) Osmic acid colours the fibrils; not a grey tone it is true, but this may be explained by the very small amount of medulla present.

(2) Weigert's hæmatoxylin stain for medulla gives a blue tone to this structure.

(3) The fibres contain neuro-keratin as they are not digested by trypsin at 40° C.

In the above account of fibres of Remak, Boveri makes a very great advance in giving good figures of transverse sections, and in maintaining that the nuclei are embedded in a sheath. But, owing to a desire to bring all nerve fibres into uniformity, he is led into the statements which, as I shall try to show, are erroneous, that the sheath of a fibre of Remak is medullated and that after staining a transverse section with acid fuchsin, each fibre has a central red dot, like the axis cylinder of a medullated nerve. And, further, he does not explain how it is that a fibre of Remak is often seen to be broken up into fibrillæ, as Key and Retzius described, and as undoubtedly can always be seen on teasing a non-medullated nerve.

This appearance is so distinct that it led Key and Retzius, as we have seen, to regard a fibre of Remak as consisting of a bundle of the finest fibrillæ, to which opinion Schiefferdecker also strongly inclines[19]. This author considers that the above mentioned fibrils are by no means to be confounded with the axis cylinders, formed by fibrillæ,

of medullated fibres, but that, with this admission, a fibre of Remak may be said to consist of a bundle of axis cylinders held together by an incomplete sheath of Schwann, persisting in the nuclei, which appear to be associated with some sheath-like element as, wherever the nuclei occur, there is a compression of the axis cylinders of the fibre of Remak[1]. He says these axis cylinders or fibrils are only 1μ in diameter, but he does not give the diameter of a fibre of Remak. He denies that a fibre of Remak has a medullated sheath, or that Weigert's hæmatoxylin method colours it, as Boveri described; and further, he cannot admit the plexiform arrangement which Ranvier described. The incomplete sheath of Schwann, he thinks, must be derived from the sheath of the ganglion cells from which the fibrils spring.

Lastly, Kölliker[13], in his text-book, agrees almost entirely with Schiefferdecker's account, and, so imbued is he with the idea that fibres of Remak consist of bundles of fibrils, all packed closely together, that he says, a transverse section ought to appear as a surface homogeneously dotted. However, his figures of transverse sections either show nothing at all distinctly, or else, open spaces surrounded by dots (Fig. 1): and this latter appearance, he says, is usually obtained. His interpretation of it is that through the reagent diffusing in, the fibrils of a fibre of Remak get pushed away from one another in such a way that they leave a cylindrical open space between them.

He contradicts Ranvier by asserting that acetic acid (8 drops to 100 c.c.) does not cause the fibrils to swell up, and that this is a good means of distinguishing them from connective tissue, which, again, he says, can be done either by treatment with concentrated potash, as this causes the fibrils to break up and dissolve at a time when connective tissue is unaltered, or by boiling, for the fibrils forming the fibres of Remak do not dissolve like connective tissue fibres, but only coagulate.

He agrees with Schiefferdecker in regarding the fibrils as equivalent to naked axis cylinders, so that fibres of Remak are but bundles of these[2]. He does not, however, describe an incomplete sheath in quite the same way as Schiefferdecker, but says that he has isolated nuclei with a short cell body at each end, and that the meaning of this is that the nuclei have cell-bodies with processes which wrap round the fibre just where the nuclei occur. He gives the

[1] See Fig. 113 in his *Gewebelehre*, p. 186. 1891.

[2] See Fig. 354, p. 34 in his *Gewebelehre*.

diameter of fibres of Remak as 4μ; the length of nuclei as 12·20μ; the diameter of fibrils as 1μ.

I have given the views of these last two authors rather more fully than I should have done, had I not thought that they represented the ideas most widely held at the present day, with regard to the structure of fibres of Remak. But, after all, it may be seen, from the foregoing account, that the following are the only facts upon which all authors are agreed.

(1) That non-medullated nerves may be teased into bands having a diameter of 1·6μ to 4μ showing a longitudinal striation on their surface, and having a great tendency to break up into fibrils of about 1μ in diameter.

(2) That there are nuclei 12·20μ in length which are generally described as situated on the surface of these bands (Max Schultze, Key and Retzius, Ranvier, Schiefferdecker and Kölliker), but sometimes as embedded in the bands (Boveri).

But further than this, all is obscure, so that the following are the questions I hope to be able to answer to some extent.

(1) Does the fibril noticed by many authors really exist at all, or is it only an artificial product of teasing?

(2) If these fibrils exist, is a fibre of Remak which is a bundle of such fibrils, to be considered as the ultimate element rather than the fibrils?

(3) How are the appearances seen in transverse sections of non-medullated nerves, such as Fig. 1, to be explained, if fibres of Remak do not consist of bundles of fibrils closely placed together?

(4) What is the connection between a fibre of Remak and the characteristic nuclei accompanying it? And is there any sheath, complete or incomplete, present?

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Part II. Normal Structure and Post-Mortem changes of fibres of Remak.

Having thus given above the various views held as to the structure and nature of fibres of Remak, and also the points of enquiry to which the same lead, I shall most fitly introduce my own observations by describing the appearance and size of fibres of Remak in the fresh condition. If a piece of the splenic nerve of an ox, or the internal carotid branches of a rabbit's superior cervical ganglion, are taken from the body as quickly as possible after death, and then teased in a little aqueous humour or iodized serum, there are seen fibres having a diameter of about 1·5 to 2μ, with nuclei along their course (Fig. 2). These obviously correspond to the fibres described by Remak, and so in the rest of this paper I shall consider that a fibre of Remak has a diameter of 1·5 to 2μ or a little more. The fibres can be seen to be slightly granular, and to swell out somewhat where the nuclei occur, which can only just be seen as pale, ill-defined structures about 16μ long and 4μ in diameter.

Having thus determined the size of fibres of Remak, it will be convenient to consider the appearances that are obtained in transverse sections of non-medullated nerves; as thereby we can determine, once for all, whether fibres of Remak consist of and appear as little groups of fibrils, each group having a diameter of 1·5 to 2μ, or, whether they exist and appear as well defined structures with sharply limited outlines.

To obtain a really good transverse section of non-medullated nerves, it is necessary to take a small piece of nerve from the body as soon after death as possible, and to fix it with osmic acid of at least ·5 per cent. strength; but, better still, with osmic vapour followed by harden-

ing in Flemming's fluid, as there is not then the same liability to shrinkage as there is by using the acid itself. Figure 3a is a drawing from a section of a piece of splenic nerve, so treated and stained with hæmatoxylin. Here and there are nuclei scattered about, but everywhere may be seen well-defined circles, with a deeply coloured circumference and a centre but slightly stained. They are all uniformly of the same diameter, namely, about 1·5 to 2μ. Therefore each circle must be the section of a fibre of Remak, consequently a fibre of Remak consists of two parts, a less deeply staining core, and a more deeply staining outer part[1].

But if a fibre of Remak in transverse section shows such a distinct structure and well-marked outline, it may well be asked, How is it that Key, Retzius, Schiefferdecker and Kölliker have all described fibres of Remak as consisting of bundles of fibrillæ? And, undoubtedly, in teased preparations of hardened non-medullated nerves, the fibres of Remak are nearly always seen to be breaking up into fibrillæ, as may be seen in Figs. 4a, 4b, 6. Further, in transverse sections, a dotted fibrillar appearance is of very frequent occurrence: such an appearance is shown by Fig. 1.

In fixing a piece of non-medullated nerve, two considerations must be borne in mind: (1) that the nerve be as fresh as possible; (2) that a suitable fixing reagent be employed.

First, then, I have found that if a piece of the splenic nerve[2] of an ox be put into a fixative reagent within ten minutes of death (this means putting the nerve into the reagent at the slaughter-house itself) then, no matter what the reagent be, if its fixative power be great enough, the appearance in transverse section will be *more* characterized by the presence of circles such as I have described in Fig. 3, than by the presence of dots; though it is true that with any other reagent but osmic acid, the circles will not be nicely defined and of uniform diameter, but distorted. Figs. 7, 8, and 11 show such appearances.

Secondly, if a piece of splenic nerve is taken from a spleen while still warm after death, but not till some 30 minutes or longer have elapsed (which is usually the case if the nerves are not taken from the spleen till it has been brought from the slaughter-house to the laboratory) and is then put into a fixing reagent, it is more than

[1] With all methods of staining hardened non-medullated nerves, I find that the outer part of the fibre stains as deeply as the core and generally more deeply.

[2] I take a splenic nerve, as it is the nerve from which non-medullated fibres are usually obtained, and which is figured by nearly all authors.

probable that a transverse section will show an appearance of dots, as in Figs. 1, 11, and 12.

The process that has been taking place can be beautifully seen, if rather a large piece of nerve is taken and put into ·5 per cent. osmic acid. For here, if a transverse section be made, all around the outside of it will be seen circles of uniform diameter, such as have been described in Fig. 3, more towards the centre of the section will be seen the same circles, but some much larger while others are much smaller, till they appear almost as dots.

And, lastly, at the centre of the section will be seen the result of this process carried on one stage further. For here some of the circles have got so large that they have burst, the outer part of the circles which stains deeply breaking up into fine fibrils, while the inner part staining but slightly is not very apparent, but can, nevertheless, be seen to be mixed up with the fine fibrils into which the outer part of the fibres has broken. Besides these fine fibrils will also be seen nuclei and large dots which are the circles which have become smaller. In other words, a post-mortem change under the influence of the reagent has taken place. The fibres of Remak have been properly fixed at the outside of the piece of nerve. Further in, where the reagent has taken longer to penetrate (*i.e.* in weaker solutions with less fixing power), the fibres have become varicose, and in the centre of the nerve the fibres have burst at the varicosities, and here the outer parts of the fibres have split into fibrils letting the core escape, while between the varicosities the fibres have shrunk so as to appear as rather large dots in transverse section. Figs. 9, 10, 11, 12, show this process in its progressive stages. All this is, in fact, nothing but the post-mortem changes which take place in non-medullated nerves; and that this is the right interpretation, is clearly shown by two other methods of staining the nerves.

1. If a large piece of nerve be treated by Golgi's silver chromate method, or rather Ramon y Cajal's double impregnation variety of it, and longitudinal sections be made of the same, then the fibres of Remak come out as most distinct and sharply limited, but, at the same time, most decidedly varicose structures. Some of the varicosities may be as large as 5μ. Fig. 5 is from such a section.

2. If, again, a piece of nerve be teased in a single drop of ·06 per cent. solution of methylene blue in ·6 per cent. salt solution, then many of the fibres are seen to be markedly varicose, as is shown in Fig. 13 a.

From all these appearances then, it is certain that through diffusive changes taking place in the dying tissue, the fibres of Remak swell up in places so as to become varicose; or, rather, that the part which stains with methylene blue, which, as I shall show shortly, is the core of the fibres, becomes varicose while the outer part of the fibres mechanically follows the excursions of this inner part. Consequently Fig. 1 or 12 is but the appearance which a piece of nerve that has undergone extreme post-mortem changes, shows in transverse section.

We can now explain why fibres of Remak after a fixing reagent, when teased out, so often break into fibrillæ. But before commencing it is necessary to bear in mind the difference in staining properties of the outer part and core of a fibre of Remak. It will be found that the fibrillæ into which the fibres in teased preparations break, have the staining properties of the outer part of the fibre. This is just what would be expected from the appearances seen in transverse sections of a piece of nerve undergoing post-mortem changes; and, as a matter of fact, many of the preparations of nerves showing fibrillar fibres of Remak, may be explained in this way. Again, it can be seen from transverse sections (Fig. 3) how closely the fibres of Remak adhere to one another; consequently, if a non-medullated nerve be teased after being fixed, the mere mechanical teasing apart of the fibres with the points of the teasing needles greatly assists in giving them a fibrillar appearance.

But it is also true that if a piece of fresh nerve be carefully teased out in aqueous humour and crushed under a cover slip and then fixed immediately with 1 per cent. osmic acid, the fibres of Remak will still *often* have a fibrillar appearance (Fig. 6). If these fibres, however, be stained with warm fuchsin for 15 minutes, and then stained with hæmatoxylin to bring out the nuclei, there will be seen besides the deeply stained nuclei and fibrils, an almost colourless structure with a granularity like ground glass about 1μ or less in diameter, running between the fibrillæ. This is, in fact, the inner part or core of the fibre.

Hence it is clear that the outer part of the fibres has a great tendency to split into fibrils, especially if this tendency is helped mechanically by teasing the fibres apart or by crushing.

On the other hand, fibres can sometimes be seen which have not split up, and in this case they are found to be cylindrical and to have a diameter of 1·5μ to 2μ, whereas, when they split into fibrils, the fibrils spread out and give the fibre a flattish appearance and an apparent

diameter of 3μ or even 4μ[1]. This appearance of intact cylindrical fibres of Remak is shown in Fig. 14 and should be contrasted with the crushed fibres shown in Fig. 6.

This Fig. 14 brings me at once to the question of the nuclei, for here there can be no doubt that the nuclei are inside or embedded in the outer part of the fibre. This is seen here so clearly because the nuclei have been very lightly stained. If, as is usually the case, they are stained deeply, as in Fig. 6, they come out so much more prominently than the rest of the fibre that they stand out and appear to lie on the surface of the fibre.

The question now arises: With what part of the fibre are they connected? With the inner core of the fibre of Remak, or with the outer part which stains well, and readily splits into fibrils? There can be no doubt that they are in connection with the outer part of the fibre, and that the two together form a sheath for the inner core which is the process of a nerve-cell and carries the nervous impulse.

The reasons for this view, which are as follows, prove at the same time that the part of a fibre of Remak which stains with methylene blue is the core.

(1) If a nucleus be detached, more often than not ragged fibrils from the outer part of the fibre are seen clinging to it.

(2) When a piece of nerve is stained with a solution of methylene blue in aqueous humour, the fibres which appear are uniformly cylindrical, with a diameter of about 1μ (Fig. 13 b), and they show no swellings indicating nuclei such as do the fibres in Fig. 14. Therefore the nuclei must be outside this fibre staining with methylene blue, which must, consequently, be the core of a fibre of Remak.

(3) When the fibres of Remak are brought out by Golgi's method (Fig. 5), or by a solution of methylene blue in ·6 per cent. (Fig. 13 a), salt solution, varicosities appear on them as we have seen. But these varicosities are of such a size and shape that they could not contain the nuclei. Therefore the nuclei must be outside the core of the fibre in which is deposited the silver chromate precipitate.

(4) A transverse section of fibres of Remak shows that the nuclei are in connection with the outer part of the fibres rather than with the inner core, for the outer part of the fibre is sometimes seen to proceed from the edges of the nucleus over the core, which often makes a groove in the nucleus (Fig. 9), as it passes by it.

[1] Cf. Key and Retzius' description of fibres of Remak (p. 270 *supra*).

(5) If, as Ranvier directed, a piece of splenic nerve be left in ammonium bichromate for several months, then the fibres of Remak are found to be very varicose: if now they are stained for two days with hæmatoxylin, the nuclei and the outer part of the fibres are stained to excess; that is, have become almost black, while the core has at length after two days taken up a certain amount of stain and come out fairly distinctly (Fig. 15). It is now found that the core is varicose and quite independent of the nuclei, while the outer part of the fibres, much broken into fibrillæ, adheres to the nuclei. Now, the characteristic varicose fibres which are brought out by staining non-medullated nerves with ·06 per cent. solution of methylene blue in ·6 per cent. salt solution, are exactly alike, in shape and size, to the core of fibres of Remak which is shown so distinctly alongside its fibrillar sheath, in a preparation from a piece of nerve hardened for several months in ammonium bichromate and then stained for two days in hæmatoxylin.

(6) As I shall show in the second part of this paper, when non-medullated nerves are cut off from their trophic nerve-cells in a ganglion, then they degenerate, as is shown by their loss of irritability. Now the only histological change that I have found is: that methylene blue no longer brings out the fibres so characteristic of non-medullated nerves, while in teased preparations, after osmic hardening, the core of the fibres is seen first to become granular and then to disappear. But the nuclei and walls of the fibres seem to be quite unchanged and as normal as ever.

From all these facts, but the latter two especially, it is proved that the core or inner part of the fibre stains with methylene blue; that it carries the nervous impulse and is the process of a nerve-cell, while the outer part is in connection with the nuclei and merely forms a sheath for the cell-process.

It will thus be seen that Key and Retzius, Schiefferdecker and Kölliker have all mistaken the fibrils, into which the sheath of the fibre so easily breaks, for axis cylinder processes, and, in consequence, they have been unable to explain the presence of the nuclei that accompany fibres of Remak, and so have had to invent incomplete sheaths of Schwann, cell bodies for the nuclei, etc. to account for their presence.

The reason for this error is very natural, as (1) the core through its poor staining properties is seen but with difficulty in teased preparations; and (2) is not seen in transverse sections if post-mortem changes have taken place as is usually the case if a splenic nerve is not put into

the fixing reagent at the slaughter-house, but is only taken from the spleen still quite warm after death, when it has been brought to the laboratory. And it is noteworthy that the figures of transverse sections given by Key and Retzius, and Kölliker all show post-mortem changes. (3) Lastly, the depth with which the nuclei stain, makes them appear generally on the outside of the sheath which (4) between the nuclei so readily splits into well-staining fibrils.

It is now possible to answer the questions which started us on these observations and which may be found on page 276.

Answering them in order:

(1) The fibrils noticed by many authors do not exist at all, but are only artificial products of teasing.

(2) Consequently a fibre of Remak is not a bundle of fibrillæ. In reality it consists of an outer wall which stains well with ordinary staining methods and an inner core which hardly stains at all, but is made very conspicuous by being stained in the dying state by weak methylene blue solutions. Hence a fibre of Remak is as much the unit of non-medullated nerves as a medullated fibre is the unit among medullated nerves.

(3) When a transverse section shows a number of dots which are apparently the sections of fibrils, it is the result of post-mortem and diffusive changes which have taken place in the nerve. A good transverse section shows all the fibres of Remak as well-defined circles of uniform diameter viz. about 1·5 to 2μ. The circumference of the circle corresponding to the wall of the fibre stains well with hæmatoxylin and other stains, whereas the centre or core stains very indifferently.

(4) This outer wall forms a sheath for the core or cell-process and the nuclei are embedded in, and form part of, this sheath.

There are two more questions which might now well be asked:

(1) What is the minute structure and chemical nature of the core and sheath of a fibre of Remak?

(2) What is the relation of the fibres of Remak to other nerve fibres?

The account of the fibres of Remak which I have just given resembles in some important respects that given by Boveri. But it proves, more conclusively as I think, that fibres of Remak consist of a nucleated sheath enclosing a core, and further explains, which he did not do, how it is that fibres of Remak have generally been described as bundles of fibrillæ. When, however, the question of the nature of the core and sheath arises, I must totally differ from Boveri. He

maintained, it will be remembered, that a fibre of Remak was exactly like a medullated fibre, the sheath being a sheath of Schwann partly consisting of myelin, and the core exactly like an axis cylinder. He made this assertion because he said that in transverse sections of non-medullated nerves, stained by Kupffer's acid-fuchsin method, the outer parts of the fibres were almost unstained, while a distinct dot appeared in the centre of each fibre. Further, he maintained that the sheaths of the fibres contained myelin, because he said they were coloured blue by Weigert's hæmatoxylin method, and were also stained by osmic acid.

However, although osmic acid does unquestionably colour the sheaths of the fibres slightly yellow, it does not give them the grey tone so characteristic of myelin: in addition to which I quite agree with Schiefferdecker that Weigert's hæmatoxylin method does not give the fibres the blue tone characteristic of myelin. And, since the researches of Gad and Heymann[1], there can be but little doubt that non-medullated nerves do not contain myelin.

Next, with reference to the core of the fibres, I have tried Kupffer's acid-fuchsin method several times and I always find that the sheaths of the fibres are stained quite as deeply as the dots which appear in the core of the fibres. But what is still more striking, a great number of the fibres do not show any dots in the centre at all: others show two, three, or even four dots, while others, instead of dots, exhibit a sort of ill-defined granularity (Fig. 18).

These appearances, in fact, correspond to the granular appearances of fibres of Remak, teased out in iodised serum (Fig. 2). For the core of the fibres is but a process of a nerve-cell, consisting of rather fluid undifferentiated protoplasm, which is probably granular or, at least, under the influence of osmic acid becomes granular, since nerve-cells after osmic acid hardening are full of granules.

If, then, a fibre of Remak is cut in transverse section at a place where there are one or more granules, these will be coloured by the fuchsin and will appear as dots in the core of the fibre. If it is cut, where there are no granules, the core will only appear slightly pink and will contain no deeply coloured dot or dots.

Further, when the core of the fibres is brought out with methylene blue, there is no indication of fibrillation, but rather a distinct appearance of granularity. And, lastly, if a transverse section be stained with

[1] *Du Bois Reymond's Archiv für (Anat. und) Phys.* 530. 1890.

hæmatoxylin, which is an admirable axis cylinder stain, the core stains so slightly that it cannot be said to have the staining properties of an axis cylinder.

This, then, raises the question of the relation of fibres of Remak to other nerve fibres. In so far as they consist of a sheath, enclosing the process of a nerve-cell, they are like medullated fibres: but the same might be said of the nerve fibres of the Crustacea. And, in fact, the fibres of Remak are almost identical with the latter, at any rate in transverse section; as will at once appear, if my figures be compared with those given by Hardy[1], in his paper on the Nervous System of Astacus. Perhaps in the latter, fewer nuclei are seen and certainly some of the fibres of Astacus are very much larger than fibres of Remak, being as much as 30—60μ in diameter, and their core seems to be very much more fluid and to contain fine fibrillæ. At the same time it is worthy of notice that both fibres of Remak and the nerves of crustacea can only be properly fixed by using osmic acid and vapour, because the cores of both are sufficiently fluid, that if the fibre be not immediately fixed, diffusion and other changes quite distort the outlines and appearance of the fibres.

With regard to the relations of fibres of Remak to the axis-cylinder of medullated fibres, I have not much to say, although nearly all authors compare the two structures fairly fully. But such a comparison is rather vain, till the structure of the axis-cylinder of medullated fibres is better known. Thus, while Ranvier describes it as an homogeneous rod, Max Schultze and Engelmann describe it as a bundle of fibrillæ; and this is not surprising when we consider that any fixing reagent must take some little time to penetrate through the medulla and that the axis-cylinder, if it be at all like fibres of Remak or the nerves of Crustacea, must be a delicate structure.

At the same time I think that it is worth while to point out a few striking facts, without commenting upon them.

(1) I find that by carefully fixing medullated nerves with osmic acid, I can get, if the axis-cylinder does not shrink, an appearance such as is figured by Schäfer in Quain's *Anatomy*, Vol. 1, on page 314. Here the appearance is very like a transverse section of fine fibres of Remak: that is, it seems to show a section of a number of tubules. Further, the borders of the tubules have staining properties similar to the sheaths, while the centres of the tubules have staining properties similar to the core of fibres of Remak.

[1] *Phil. Trans. of the Roy. Soc.* 1894. B. Plate XI. Figs. 14 etc.

(2) As I have shown that post-mortem changes in fibres of Remak result in the formation first of varicosities and then of a number of dots, so might the dotted appearance often given in transverse sections of the axis-cylinder of medullated nerves be explained, since the fibrillæ, into which the axis-cylinder undoubtedly divides up at its termination, are often characterised by their varicose appearance.

(3) Joseph[1] maintained that Kupffer's acid-fuchsin method stained some supporting sheath-like element in the axis cylinder and not the nerve-cell process itself. And certainly it does seem that the sheath, and not the core, of fibres of Remak has similar staining properties to those of the axis cylinder of a medullated nerve. Thus the following special axis-cylinder methods: Freud's[2] gold potash, Palladino's[3] palladium chloride, Kupffer's acid fuchsin, Nissl's[4] congo red, Stroebe's[5] bleu de Lyons, all stain deeply the sheath of fibres of Remak and not the core.

(4) Kühne and Steiner[6] noticed that ·1 p.c. osmic acid made something swell up in the axis cylinder of medullated nerves and had also the same effect on fibres of Remak. This I can confirm, as I find that ·1 p.c. osmic acid does make the core of fibres of Remak swell up, because such a strength of osmic acid has very little rapid fixing power.

(5) There is undoubtedly a neurokeratinal element in the medulla and probably also in the axis cylinder of medullated fibres. Now, the sheath of fibres of Remak seems also to consist of neurokeratin, since I can confirm Boveri's[7] statement, that trypsin at 40° C. does not digest the sheath of fibres of Remak.

This brings me to the consideration of the chemical nature of the sheath of a fibre of Remak. That it is allied to neurokeratin is further suggested by the fact that it does not contain myelin, and has not the staining properties of a sheath of Schwann, whereas it has very much the same staining properties as the neurokeratin network in the medulla of a medullated nerve.

Lilienfeld and Monti's method[8] distinctly makes it dark, so that if the method is to be trusted, it contains phosphorus.

[1] *Archiv für (Anat. und) Phys.* 1888. 184.
[2] *Archiv f. Anat. (u. Phys.)* 1884. 453.
[3] *Zeit f. wiss. Mik.* VII. 237. 1890. Also *Journ. Roy. Mic. Soc.* 1890, 817 and 1892, 439.
[4] *Zeit. f. wiss. Mik.* III. 298. 1886.
[5] *Centralbl. f. allgem. Pathol. u. pathol. Anat.* V. 49–57. 1893.
[6] *loc. cit.* 166.
[7] *loc. cit.* 488.
[8] *Zeit. physiol. Chem.* XVII. 410. 1893.

Lastly, it is unaffected by acetic acid, whereas it is easily dissolved by caustic potash.

Before concluding, we must shortly consider the origin of fibres of Remak from nerve cells; and it may be also worth while to repeat the dimensions of the various parts of a fibre.

The diameter of the core of a fibre of Remak is 1μ or a little more, while the fibre itself has a diameter of 1·5μ to 2μ, if it is cylindrical and intact. But in teased preparations, when the sheath breaks into fibrillæ, the fibres may appear to have a diameter of 3 to 4μ. The fibrils, into which the sheath breaks, vary from anything extremely fine to as much as ·5μ. The nuclei are usually 10 to 16μ long and 3 to 4μ in diameter. These figures refer chiefly to fibres taken from the splenic nerve of an ox, as the fibres of the branches of a rabbit's superior cervical ganglion seem often to have a smaller diameter.

With reference to the origin of fibres of Remak from sympathetic nerve cells, there seems to be no doubt from the researches of Kölliker[1], Ramon y Cajal[2], van Gehuchten[3], and Sala[4] with Golgi's method, and of Dogiel[5] with methylene blue, that of the processes of the multipolar nerve cells in the ganglia of the sympathetic system [with the exception of the ganglia of the plexus of Auerbach and Meissner, and of what Ramon y Cajal calls interstitial ganglia], some are protoplasmic processes or dendrites while others are axis-cylinder processes which become so-called fibres of Remak. Further, apart from Kölliker's account and the earlier views of Ramon y Cajal, there seems to be an unanimous opinion that each nerve cell has only a single one of these nervous or axis-cylinder processes, although it may have as many as twenty protoplasmic processes. The characters of this axis-cylinder process are very similarly described by all the above authors. Briefly they are as follows. It is of a fair size, homogeneous in appearance, and possesses in its whole course a uniform diameter. It is essentially undivided and according to van Gehuchten and Sala devoid of collaterals, though Dogiel maintains the existence of some extremely fine collaterals: but it is characterised by varicosities which more or less large and abundant give to certain portions of the process a moniliform appearance.

[1] *Sitzungs-Ber. d. phys.-medic. Gesellsch. zu Würzburg.* Oct. 1889. 166.

[2] *Notas preventivas—II. Extraido de la Gaceta Sanitaria del 10mo Dec.* 1891: *Comptes rendus hebdom. des Séances de la Soc. de Biol.* 5th Jan. 1894.

[3] *La Cellule*, VIII. 86. 1892.

[4] *Archives Italiennes de Biologie*, XVIII. 439. 1893.

[5] *Archiv f. mik. Anat.* XLVI. 305. 1895.

Such a description harmonises in every way with my account of the core of fibres of Remak. For I have never seen the latter divide either in the splenic nerve of the ox after treatment with Golgi's method or with methylene blue, or in the branches of a rabbit's superior cervical ganglion treated with methylene blue. Consequently each fibre of Remak in these nerves is in connection with a separate sympathetic nerve cell, and runs a long undivided course until it divides at its termination.

The differentiation of these axis-cylinder processes or as we may now call them, the cores of fibres of Remak, from medullated fibres ending in sympathetic ganglia is still uncertain, since Dogiel emphasises the greater varicosity of the latter as the chief difference between them while on the contrary it is the absence of varicosities in the latter, together with extremely fine branching collaterals that Sala takes to be the notable difference.

From protoplasmic processes or dendrites the axis-cylinder process seems to be differentiated by the three following characteristics of the former. 1. Shortness of course. 2. Frequency of division. 3. Irregularity of form. This latter characteristic Dogiel especially has mentioned, describing numerous minute side processes as appearing like so many little thorns on the course of the dendrites. At the same time I think it probable that these short bifurcating dendrites are formed by prolongations of cell substance just like fibres of Remak since both are equally varicose and of the same size in the figures given by the above mentioned authors who employed Golgi's method. However it is possible that, as regards a sheath, the dendrites differ from fibres of Remak, since Dogiel denies the existence of nuclei along their course.

The varicosities shown by Golgi's method are due as I have already pointed out to a swelling of the core of the fibres, as the result of diffusive changes acting on dying fibres. Then the silver chromate precipitate becomes deposited in the varicose core of the fibres, so that Golgi's method only brings out the core, or nerve cell processes themselves.

The sheath of the fibres must be derived from the sheath of the ganglion cells, a fact which Valentin, as we have seen, emphasised and which misled him into thinking that fibres of Remak were only of a connective tissue nature. He said that fibres of Remak were prolongations of the capsules of the ganglion cells, and this view is probably right as regards the sheath of fibres of Remak.

I may finally point out that the conception that fibres of Remak

have a core which is but a prolongation of the granular contents of a nerve-cell, is in harmony with the generally accepted view of the nature of cells. For just as the nutrition and life of the protoplasm of any part of a cell depend on connection with its nucleus, so the life of the protoplasmic contents of a fibre of Remak will depend on connection with the nucleus of its nerve-cell. And, lastly, if nervous impulses have a physical and not a chemical explanation, as is, on the whole, very possible[1], this tubular structure of fibres of Remak lends support to the conception of the nervous impulse being transmitted by changes in the surface tension of tubular canals.

Conclusions from Part II.

It has been shown:

(1) That a fibre of Remak is a structure, having a diameter of 1·5 to 2μ, consisting of an outer sheath, enclosing an inside core.

(2) That this core is the process of a nerve-cell, while the sheath is in connection with the nuclei and is probably derived from the capsule of a nerve-cell.

(3) That in transverse sections, fibres of Remak should all appear as tubes of uniform diameter, and not show any dots at all.

(4) That if a transverse section shows a dotted appearance, this is the result of post-mortem and diffusive changes having taken place in the nerve.

(5) That such post-mortem changes consist in the core of the fibres swelling up so that the fibres become varicose, until eventually the varicosities burst and the sheath of the fibres breaks into fibrillæ.

(6) That as the result of such a process taking place in conjunction with a natural tendency of the sheath to break up, fibres of Remak, in teased preparations, have usually been described as bundles of fibrillæ.

(7) That this mistake is furthered by the fact that the core of the fibres of Remak stains but very slightly with ordinary staining reagents; so that it has hitherto been overlooked by all authors but Boveri.

(8) That this core can be shown very distinctly by either of three methods: (i) by Golgi's method, (ii) by the action of methylene blue on fresh fibres, (iii) by staining with hæmatoxylin for two days nerves which have been hardened several months in ammonium bichromate; but this core is not exactly like the axis cylinder of a medullated fibre.

[1] Edes. This *Journal*, XIII. 431. 1892.

Olfactory Nerve Fibres.

Since Max Schultze, Key and Retzius and others have asserted that olfactory nerves and non-medullated nerves from the sympathetic system of vertebrates consist of similar fibres, I thought it would be unnecessary to give any observations of my own on olfactory nerves. However on making transverse sections of nerves from the olfactory groove of the pike, and also of the olfactory nerves of the sheep and rabbit just where they enter the cribriform plate of the ethmoid, I could not convince myself of the truth of such a statement. The nerves from both animals I have studied after fixation in osmic vapour, 1 per cent. and ·5 per cent. osmic acid; saturated aqueous solutions of corrosive sublimate; and saturated aqueous solutions of picric acid. In these transverse sections I can confirm the existence of Max Schultze's primitive olfactory nerve fibres. But as far as I can see, the nuclei present belong chiefly to the sheath of the same. The contents of these fibres do not seem to me to consist of fibres similar to fibres of Remak: but rather of a bundle of nerve-cell processes, each being about ·5μ or a little less in diameter, since I find that treating the olfactory nerves of a rabbit or pike with methylene blue brings out axis cylinders of about this diameter. These axis cylinders do not seem individually to have a separate sheath, but rather they penetrate a common sheath which is derived from the sheath of the olfactory fibres, and forms with the axis cylinders the contents of the same. Belonging to this sheath inside the olfactory fibres are a few nuclei which for the most part do not resemble the nuclei of fibres of Remak. A somewhat similar description of olfactory nerve fibres has already been given by Boveri[1], who also could not confirm Max Schultze's account of olfactory fibres being constituted by fibres of Remak. But Boveri regarded the sheath filling up the olfactory fibres as containing myelin, which has since been denied by Gad and Heymann[2].

I have not yet had time to make further investigations into the structure of olfactory nerve fibres, though perhaps at some future time I shall do so. However, in spite of this, it seems to me worth while to make these few remarks, so as to point out that the above conclusions concerning the structure of fibres of Remak are not as yet applied by me to olfactory nerve fibres, although they also are non-medullated.

[1] *loc. cit.* 489. Also see Figs. 21 and 22, Pl. II.

[2] *Archiv für (Anat. u.) Phys.* 530. 1890.

Part III. Degenerative Changes.

Having now got some idea of the structure and normal appearance of fibres of Remak, it will be most convenient to take up the histological changes, before dealing with the physiological phenomena that occur in degeneration.

Histological Changes.

As far as I know, the only previous observation on the subject is a slight account given by Langley and Anderson[1] of the changes which occur in the hypogastric nerve of the Cat. They say: "The non-medullated fibres show no striking evidences of degeneration. The fibres in the peripheral end of the cut nerve seem simply to dwindle: occasionally they become finely granular, but commonly we have not found this. In teased specimens they break up into very delicate elongated spindle-shaped filaments: in transverse section even after ten days of degeneration, they differ very little from normal fibres." This account does in fact summarise the changes, or rather absence of changes, that are shown by ordinary staining methods: so much so that it is often very difficult to tell by their appearance whether fibres of Remak are normal or degenerated. But, as I shall show, by the use of methylene blue it is possible to demonstrate a distinct change and so to unhesitatingly distinguish normal from degenerated non-medullated nerves. It will be convenient, however, first to describe the methods I have employed, and also the nerves on which I have worked.

It was important that the nerves I studied should consist purely of fibres of Remak or at least contain them in preponderating numbers, so as to admit of no chance of their being confused with very fine medullated nerves. At the same time it was desirable both that they should be easily cut, and that some marked effect should be the result of their degeneration. I therefore have worked entirely on the branches of the superior cervical ganglion in the rabbit, and, chiefly on those which run with the internal carotid artery through the carotid canal in the base of the skull, to reach finally the iris and some other structures. Their physiological effect is notably that of causing dilatation of the pupil.

Their fineness however constitutes a difficulty: for, owing to it,

[1] This *Journal*, XVII. 183. 1894.

and the tendency of osmic vapour or osmic acid used sufficiently strong to produce immediate fixation, to cause considerable shrinkage, I have been unable with certainty to obtain transverse sections of normal nerves which showed the outlines of the individual fibres with sufficient clearness. Only occasionally have I been successful (Fig. 17). Therefore I consider that till I am able with certainty always to obtain intelligible transverse sections of these nerves when normal, transverse sections of them when degenerated are worthless. At the same time, as far as I can judge, transverse sections of degenerated nerves of which I have made a considerable number, do not show any striking changes.

In this connection also I must mention my inability to successfully employ Golgi's method on these nerves. I have tried again and again on the branches of the superior cervical ganglion of rabbits and cats, with and without the use of gelatin, but with no success. It requires a large piece of nerve such as some of the largest pieces from the splenic nerve of the ox, to obtain the appearance of fibres so characteristically varicose by this method. And then they are only brought out in the very centre of the piece of nerve. Consequently all my observations of degeneration are from teased preparations. For this purpose, the nerves have always been taken from the carotid canal as quickly as possible after bleeding the rabbit to death: sometimes also from the internal carotid branches outside the canal. But the latter do not give such reliable appearances if they have previously been repeatedly stimulated and so exposed for some little time. The nerves, as I have said, run in the loose connective tissue surrounding the internal carotid artery, from which I carefully separate the nerve bundles, three or four in number, in a drop of aqueous humour. I then proceed to examine them in the following ways.

Methods. (1) Part I tease in a minute drop of a ·06 per cent. solution of methylene blue in ·6 per cent. salt solution: and keeping the preparation in a moist chamber, examine it from time to time to see whether any fibres of Remak appear. It is most important not to have an excess of methylene blue present; for only when this condition is fulfilled are the cores of the fibres of Remak differentiated from the surrounding structures in a really characteristic way. After an hour or more I fix the nerves in a saturated aqueous solution of corrosive sublimate. After washing out with alcohol, I stain the nerves with a solution of Delafield's hæmatoxylin saturated with chinolin and finally tease and mount in glycerine.

The result is quite good for a comparative estimate of the nuclei

and sheath in normal and degenerated nerves respectively. This method is also fair for judging of the comparative size of fibres of Remak, but it is useless for studying the core.

(2) Another portion of the nerves, I at once tease in aqueous humour, completing the isolation of the fibres by putting a coverslip on the preparation, and briskly tapping on the top of it. Then I immediately fix the nerves by running 1 per cent. osmic acid under the coverslip. In this way the fibres get isolated from one another infinitely better than if they are teased after being fixed with osmic acid. After washing the acid out in water, I stain the preparation by putting it for fifteen minutes in warm acid-fuchsin and then for two or more hours in a mixture of glycerine and Grübler's hæmatoxylin and eosin. I finally tease and mount in glycerine.

Such preparations show the nuclei, the size of the fibres, the core, and the sheath, very satisfactorily, so that it is possible, both by the size of the fibres and the granularity of the core to say whether the fibres are normal or degenerated.

(3) Another part of the nerves I have sometimes put up in 2 per cent. ammonium bichromate. After several months the bichromate has been washed out and the nerve stained for two days with a solution of Delafield's hæmatoxylin saturated with chinolin. As these nerves are so fine, the bichromate penetrates fairly quickly and so does not cause the core of the fibres to swell up to the same extent as in a fairly large piece of the splenic nerve of an ox. Still, this method shows the condition of the core and sheath of the fibres fairly well.

The various appearances given by the various methods can now be described.

Methylene Blue. Nineteen hours after section of the branches of the ganglion, I obtain an appearance as normal as ever[1]. After twenty-three hours (corresponding to the time, as will be seen, when the pupil begins to dilate and the branches of the ganglion become less irritable), I obtain almost a normal appearance, but perhaps there is a very slight diminution in the number of fibres that appear. After thirty hours there are still a few fibres brought out for some little length; but on the whole the preparation is quite characteristic, for a number of blue dots which are scattered all over the fibres, which appear to be the sole surviving remnants in their respective fibres of the core. After forty hours the method fails to bring out any fibres.

[1] Compare tabular synopsis at the end of the Appendix.

With Corrosive Sublimate. In teased preparations the nuclei may be seen to be similar to normal nuclei and the general appearance of the preparation is very similar to normal preparations. But on the whole, I have come to the conclusion that the fibres of Remak seem to consist more than ever of fibrils, and to have a smaller diameter as if something was wanting. Still I do not believe that an observer who had not just been looking at normal non-medullated nerves would be able to distinguish a degenerated from a normal preparation (Fig. 16, a, b, c, d, e).

Osmic Acid. In these preparations it is very necessary to make certain that the fibres under examination are really isolated fibres. For the treatment to which the fibres have been subjected, if it does not isolate the fibres, is very apt to make two or three run together and simulate a very broad fibre of Remak, in which condition various and very curious appearances are sometimes seen (Figs. 19 and 20). In normal fibres, properly isolated, the core is seen to be very finely granular, so as to give an appearance like ground glass. In degenerated nerves after two days the core is seen to be more coarsely granular; which appearance is characteristic of degeneration as long as any core remains. The disappearance of the core varies in different animals: sometimes I have found very little left on the fifth day; sometimes some still remaining on the twelfth day. When the core has disappeared the degenerated preparation can at once be detected by the shrunken state of the fibres; and this is the most obvious sign of degeneration (Figs. 21, a, b, c, d, e). The sheath of the fibres and the nuclei do not seem to be affected by degeneration.

Ammonium Bichromate. The difference between normal and degenerated nerves similarly consists in the presence or absence of the core.

Physiological Changes.

Phenomena are furnished (1) by the size of the pupil after the operation, up to the time of experimenting: (2) by stimulating the cut ends of the branches of the ganglion, which can be found quite easily by isolating the internal carotid artery to which they cling.

Taking the latter method first, I find that after nineteen hours the branches are still irritable. But after forty hours I can produce no dilatation of the pupil: while after twenty-three, and still more after thirty hours, the effect of stimulation is not so pronounced and a stronger current is also required. In fact, about the twenty-fourth

hour, the irritability seems to begin to depart from the branches of the ganglion outside the carotid canal, where they are capable of being stimulated. This may be due to mere traumatic changes, but I think this explanation unlikely, as these branches are perfectly irritable after nineteen hours, and the branches do not enter the internal carotid canal for nearly a centimetre after leaving the ganglion, which in an operation can be cut out very cleanly without exposing the branches more than to a minimal extent.

Further, when these fibres are stimulated quite close to the base of the skull, it is probable that some current will escape and stimulate the nerves in the carotid canal if they are still irritable.

And, lastly, experiments VII., VIII., XII., XIII., XVI., XVII. were devised so as to subject the external carotid branches of the ganglion to almost the same conditions as the cut internal carotid branches and *vice versâ*. I found that when the internal carotid branches were cut, care being taken not to injure the external carotid branches, then, after one, two, or three days, the external carotid branches were quite normally irritable, and with methylene blue stained quite normally. Similarly, when the external carotid branches were crushed with a ligature, the internal carotid branches after one, two, or three days, were found to be quite normal. In passing, I should like to mention that in these six experiments I could quite confirm Langley's[1] statement that the vaso-constrictors to the ear go both by the internal carotid and external carotid branches of the ganglion: in fact, degeneration of the internal affect the circulation of the ear much more than degeneration of the external branches; so that most of the vaso-constrictors to the ear seem to leave the ganglion by its internal carotid branches.

We may now consider the other class of phenomena, viz. the varying size of the pupil.

After an operation in which the ganglion is cut out, or the branches are cut or crushed with a ligature, the pupil on the operated side is always affected. However, the size of a rabbit's pupil varies greatly, so that in some rabbits it is very difficult to detect much difference in the mere size of the pupils of the two sides. But the following difference is always obvious, namely, that on the operated side the bottom of the pupil forms a more acute angle. From twenty-four to about seventy hours after the operation, the pupil on that side is quite as large, and often much larger than that of the normal side. This is not quite a

[1] This *Journal*, XIV. *Proceedings of the Phys. Soc.* Jan. 1893.

constant phenomenon, but I have generally observed it[1]; and I take it to be the sign of some sort of reaction of degeneration, either in the nerve or in the muscle of the iris. After three days and onwards, the pupil on the operated side is smaller and more acute at the lower border than on the normal side.

In three cases I observed that the pupil of the operated side became quite as large as that of the normal side, in Exp. XXV. two hundred and thirty-one days; in Exp. XXVI. one hundred and sixty-two; in Exp. XXVII. eighty-nine days after the operation. But in these cases, though the two pupils were equally large and the angles at the lower border of the two pupils about equal, the general shape of the pupil on the operated side was unsymmetrical. A further characteristic of these three cases was that the pupil underwent slight spontaneous movements and did not react normally to light, so that I have seen it sometimes dilate a little rather than contract on exposure to a moderate light.

In these cases I thought I should find that regeneration had occurred; but though, on stimulating the left cervical sympathetic nerve or the branches of the ganglion with a strong current, some dilation could be observed, yet it was not marked enough to make it certain that it was not due in some way to escape of current, or to mere spontaneous movements of the pupil: and more often than not stimulation had no effect. Staining the nerves with methylene blue failed to bring out any fibres of Remak, except in Exp. XXV., when a very few extremely fine fibres appeared. These may have been fibres which had just started to grow down to the iris, but had not yet reached their termination. On the other hand, in Exp. XXIV., where the pupil of the operated side was the smaller, though not unsymmetrical, I undoubtedly obtained regeneration. For stimulation, both of the cervical sympathetic nerve low down in the neck and also of the internal carotid branches of the ganglion, produced immediate and well marked dilatation of the pupil. Post-mortem examination also with methylene blue showed a very fair number of fibres in the nerves taken from the carotid canal. These fibres were for the most part extremely fine. Therefore it is obvious that mere recovery of size without symmetry of the pupil is no sign of regeneration, whereas regeneration can occur before the pupil of the operated side has become

[1] Cf. Tabular synopsis at the end of this paper. Owing to the lateral position of a rabbit's eyes, it is impossible to examine them both at once, and so it is very difficult to be certain that the position of the rabbit and the illumination of the two eyes is exactly similar during the examination of each of them.

as large as that of the normal side. The recovery of size in Exps. XXV., XXVI., XXVII., was due possibly to trophic disturbances in the muscles of the iris, since, as we have seen, they reacted abnormally to light.

In Exp. XXIV. it is to be noticed that the rabbit had been kept four weeks longer than in any other case. Consequently it is probable that in the other experiments the rabbits were not kept long enough to obtain regeneration. This explanation will most likely also apply to Exps. XXVII., XXII., XXIII., XXV., where I failed to obtain regeneration of the œsophageal fibres of the vagus [which are probably non-medullated] after 89, 125, 143, and 231 days respectively, since Vanlair[1] states that it takes at least eleven months for regeneration of the vagus in the dog to occur. Apparently the length of time necessary for regeneration of the pupil dilator nerves is not the result of interfering with the blood supply of the superior cervical ganglion when its branches are cut or crushed, since I have tried two cases (Exps. XXXI., XXXII.) of merely isolating the ganglion from the surrounding tissue; and in both cases, after eight and fourteen days respectively, the branches were both irritable and capable of staining normally with methylene blue. Further, in the case of the œsophageal fibres of the vagus, there is but little chance of disturbing the blood supply of the ganglion containing their trophic cells.

It may, finally, be worth while to contrast these results with the degeneration of medullated nerve fibres. For in the latter structure the degeneration of the medulla is the striking feature which is quite absent from the former; while, as far as the nuclei are concerned, those of non-medullated nerves do not seem to undergo proliferation like those of medullated nerves. Further, the axis cylinder of medullated nerves is said to break up completely; whereas it is only the core of non-medullated nerves that disappears.

If then the degeneration of non-medullated nerves can throw any light on that of medullated nerves, it is in the following point. In non-medullated nerves the nuclei and sheath-like structures remain unaltered, while the nerve cell processes alone degenerate. Therefore in medullated nerves, since the medulla and axis cylinder both degenerate the medulla might be a differentiated fatty outer part of the original process of a cell; and, like the axis cylinder it surrounds, dependent for its nutrition on connection with the nucleus of a nerve cell.

[1] *Archives de Phys. norm. et path.* 1894. 220.

In the rate of degeneration also, the two classes of nerves are very different. Non-medullated nerves degenerate probably by the thirtieth, certainly by the fortieth hour after cutting them off from their trophic cells, whereas many observers give four days as the average time when degeneration first appears in medullated nerves, though this varies greatly, since Ranvier has sometimes observed it occur in forty-eight hours.

Similarly, this rapidity in the degeneration of non-medullated nerves, affords a method for separating the physiological effects of medullated and non-medullated fibres in a mixed nerve. For if the nerve be stimulated two days after it has been cut, then only the effect of the medullated fibres contained in the nerve will be observed.

Conclusion from Part III.

The outcome of these few observations seems then to be that when a non-medullated nerve degenerates, the core of the fibres of Remak, staining with methylene blue, disappears, while the sheath and nuclei of the fibres are unaffected by cutting them off from their trophic nerve-cells. Degeneration is shown by the histological changes and physiological phenomena to begin about the twenty-fourth, while the loss of irritability and conductivity is complete by the fortieth hour.

The question of regeneration I have only slightly touched on, as this paper was intended chiefly for degenerative changes in fibres of Remak. However, in Exp. XXIV., after 259 days, I undoubtedly obtained regeneration. Possibly at some future time, I may publish a note on the regeneration of fibres of Remak, for which Exps. XXVIII., XXIX., XXX. are being kept alive.

In conclusion, I desire to express my sincere thanks to Mr Langley, for his valuable suggestions and criticisms.

APPENDIX.

Records of Operations and Physiological Examinations.

For cases of degeneration the superior cervical ganglion was cut out, or its branches were cut or crushed: for cases of regeneration, either the branches were cut and left as much in situ as possible, or else they were crushed with a fine cat-gut suture tied around them as tightly as possible and then cut off; since Howell and Huber[1] showed that with medullated

[1] *loc. cit.* p. 357.

nerves this method led to complete degeneration and was least likely to hinder regeneration.

Throughout the following experiments the rabbits *were always anæsthetised with ether:* my instruments were sterilised by being placed in boiling water and then put into 5 p.c. carbolic acid. All the rabbits were treated most aseptically, so that there was not a single case which did not heal perfectly cleanly. The edges of the wound were always sewn together with six to ten stitches, and then dressed with boracic cotton wool covered with collodion. In the final examination of the rabbits they were anæsthetised with A.C.E. mixture through a trachea tube and at the end of the examination were always killed by being bled to death.

Table of Experiments.

The following abbreviations are used:

L.S.C.G. and R.S.C.G. are used respectively for left and right superior cervical sympathetic ganglion.

L.C.S. and R.C.S. are used respectively for left and right cervical sympathetic nerves.

I.C.B. and E.C.B. are used respectively for internal and external carotid branches.

C.C.B. is used for carotid canal branches, viz., internal carotid branches of the ganglion which have been taken from the carotid canal instead of outside it.

lt. pl. and rt. pl. are used respectively for left and right pupil.

EXPERIMENT I. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. cut. *Examination* after 4 days: lt. pl. the smaller; animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of I.C.B. of L.S.C.G. had no effect on lt. pl.

EXPERIMENT II. Rabbit ♂. *Operation:* the I.C.B. of L.S.C.G. were crushed.

Examination after operation: lt. pl. the smaller.
" next day: lt. pl. the larger.
" after 3 days: lt. pl. the smaller.
" after 5 days: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of I.C.B. of L.S.C.G. had no effect on lt. pl.

EXPERIMENT III. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. were cut.

Examination after 7 days: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of I.C.B. of L.S.C.G. at base of skull had no effect on lt. pl.

EXPERIMENT IV. Albino rabbit ♀. *Operation:* a piece of the I.C.B. of the L.S.C.G. excised. Also the left vagus above superior laryngeal nerve cut and sutured.

Examination after 2 days: both pupils same size.

„ after 6 days: lt. pl. the smaller.

„ after 27 days: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of I.C.B. of L.S.C.G. at base of skull had no effect on lt. pl., (3) stimulation of left vagus above or below point of suture which had healed well, had no effect on heart or œsophagus.

EXPERIMENT V. Rabbit ♀. *Operation*: the I.C.B. of the L.S.C.G. were cut.

Examination after operation: lt. pl. the smaller.

„ after 19 hours: lt. pl. still the smaller: animal being anæsthetised with A.C.E. mixture: stimulation of I.C.B. of L.S.C.G. at base of skull caused good dilatation of lt. pl.

Post-mortem examination with methylene blue: fibres shown quite normally in the C.C.B. of both sides.

EXPERIMENT VI. Rabbit ♂. *Operation:* the L.S.C.G. was cut out.

Examination after 23 hours: both pupils same size: animal being anæsthetised with A.C.E mixture: stimulation of I.C.B. of L.S.C.G. at base of skull had no effect on lt. pl.

Post-mortem examination with methylene blue: normal number of fibres shown in the C.C.B. of the R.S.C.G., and also a fair number in the C.C.B. of the L.S.C.G.

EXPERIMENT VII. Rabbit ♂. *Operation:* the E.C.B. of L.S.C.G. were crushed, care being taken not to injure the I.C.B.

Examination after 23 hours: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused (*a*) dilatation of lt. pl. and (*b*) constriction of lt. ear artery, (2) stimulation of E.C.B. of L.S.C.G. with a strong current, after the I.C.B. of L.S.C.G. had been cut, caused complete constriction of lt. ear artery, (3) stimulation of I.C.B. of the L.S.C.G. caused dilatation of lt. pl. and constriction of lt. ear artery.

Post-mortem examination with methylene blue: fibres shown normally in the I.C.B. of the L.S.C.G., and also very fair number in the E.C.B. of L.S.C.G.

EXPERIMENT VIII. Grey and white rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were cut, care being taken not to injure the E.C.B.

Examination after 26 hours: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused constriction of lt. ear artery but no dilatation of lt. pl., (2) stimulation of E.C.B. of L.S.C.G. caused constriction

of lt. ear artery, (3) stimulation of I.C.B. of the L.S.C.G. with a strong current but not with a weak caused slight dilatation of lt. pl.

Post-mortem examination with methylene blue: very fair number of fibres shown in I.C.B. of L.S.C.G., while fibres in E.C.B. of L.S.C.G. were brought out normally.

EXPERIMENT IX. Rabbit ♀. *Operation:* the L.S.C.G. was cut out.

Examination after 30 hours: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: stimulation of I.C.B. of L.S.C.G. at base of skull had no effect on lt. pl.

Post-mortem examination with methylene blue: fibres in the C.C.B. of the R.S.C.G. shown quite normally: but fewer than normal in the C.C.B. of the L.S.C.G., while some fibres here and there looked as if they were breaking up.

EXPERIMENT X. Black rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were cut.

Examination after 30½ hours: both pupils same size: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of the I.C.B. of the L.S.C.G. had no effect on lt. pl.

Post-mortem examination with methylene blue: fibres shown normally in the I.C.B. of the right side: but distinctly fewer than normal in the C.C.B. of the left side; in fact the core of the fibres (staining with methylene blue) seemed in places to have begun to break up, so that only lumps of it were left here and there to be stained.

EXPERIMENT XI. Rabbit ♀. *Operation:* the L.C.B. of the L.S.C.G. were crushed.

Examination after the operation: lt. pl. the smaller.

„ next day: lt. pl. the larger.

„ after 41½ hours: both pupils same size: animal being anæsthetised with A.C.E. mixture, (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of I.C.B. of L.S.C.G. had no effect on lt. pl.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of the R.S.C.G.; but none shown in the C.C.B. of L.S.C.G., only specks of core left here and there staining with methylene blue.

EXPERIMENT XII. Rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G., were cut, care being taken not to injure the E.C.B.

Examination next day: both pupils same size.

„ after 45 hours: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused constriction of lt. ear artery but no dilatation of lt. pl., (2) stimulation of E.C.B. of L.S.C.G. caused constriction of lt. ear artery, (3) stimulation of the I.C.B. of the L.S.C.G. caused neither dilatation of lt. pl. nor constriction of lt. ear artery.

Post-mortem examination with methylene blue: fibres shown normally in the E.C.B. of the L.S.C.G.; but only a characteristic degenerated speckly appearance shown by the I.C.B. of the L.S.C.G.; no fibres at all.

EXPERIMENT XIII. Rabbit ♂. *Operation:* the E.C.B. of the L.S.C.G. were crushed, care being taken not to injure the I.C.B

Examination after 2 days: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused constriction of lt. ear artery and dilatation of lt. pl., (2) stimulation of L.C.S. with I.C.B. of L.S.C.G. cut, had no effect on lt. ear artery, (3) stimulation of E.C.B. of L.S.C.G. had no effect on lt. ear artery, (4) stimulation of I.C.B of the L.S.C.G. caused dilatation of lt. pl. and constriction of lt. ear artery.

Post-mortem examination with methylene blue: no fibres shown in the E.C.B. of the L.S.C.G.: but fibres shown normally in the C.C.B. of the L.S.C.G.

EXPERIMENT XIV. Rabbit ♂. *Operation:* the L.S.C.G. was cut out.

Examination after 49 hours: both pupils same size; animal being anæsthetised with A.C.E. mixture: stimulation of I.C.B. of the L.S.C.G. at base of skull had no effect on lt. pl.

Post-mortem examination with methylene blue: fibres shown quite normally in the C.C.B. of the right side: but none shown in the left C.C.B.

EXPERIMENT XV. Rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were crushed.

Examination after the operation: lt. pl. the smaller.

„ next day: both pupils same size.

„ after 52 hours: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. had no effect, (2) stimulation of I.C.B. of the L.S.C.G. had no effect.

Post-mortem examination with methylene blue: no fibres shown in the C.C.B. of the L.S.C.G. but quite normally in the C.C.B. of the right side.

EXPERIMENT XVI. Rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were cut, care being taken not to injure the E.C.B.

Examination next day: both pupils same size.

„ after 3 days: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused (*a*) constriction of lt. ear artery, (*b*) no dilatation of lt. pl., (2) stimulation of E.C.B. of L.S.C.G. caused constriction of lt. ear artery, (3) stimulation of I.C.B. of the L.S.C.G. had no effect on lt. ear artery or lt. pl.

Post-mortem examination with methylene blue: fibres shown normally in the E.C.B. of the L.S.C.G.: but none shown in the I.C.B. of the L.S.C.G.

EXPERIMENT XVII. Rabbit ♀. *Operation:* the E.C.B. of the L.S.C.G. were crushed, care being taken not to injure the I.C.B.

Examination after 3 days: animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. caused constriction of lt. ear artery and dilatation of lt. pl., (2) stimulation of L.C.S., after the I.C.B. were cut, had no effect on either, (3) stimulation of the E.C.B. of L.S.C.G. had no effect on lt. ear artery, (4) stimulation of the I.C.B. of the L.S.C.G. caused constriction of lt. ear artery, and dilatation of lt. pl.

Post-mortem examination with methylene blue: no fibres shown in the E.C.B., but fibres shown normally in the I.C.B. of the L.S.C.G.

EXPERIMENT XVIII. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. were crushed.

Examination next day: lt. pl. the larger.

„ after 2 days: lt. pl. the larger: animal being anæsthetised with A.C.E. mixture: the I.C.B. of both sides were put up as quickly as possible in 1 per cent. osmic acid.

Post-mortem examination with methylene blue proved that degeneration had occurred, as no fibres were shown in the C.C.B. of the L.S.C.G., but only a speckly degenerated appearance, while fibres were shown normally in the C.C.B. of the R.S.C.G.

EXPERIMENT XIX. Rabbit ♂. *Operation:* the I.C.B. of the R.S.C.G. were crushed.

Examination after 2 days: both pupils same size.

„ after 3 days: animal being anæsthetised with A.C.E. mixture, the I.C.B. of both sides were put into 1 per cent. osmic acid as quickly as possible.

Post-mortem examination with methylene blue proved degeneration, by failing to show fibres in the C.C.B. of the right side, while showing them normally in the C.C.B. of the L.S.C.G.

EXPERIMENT XX. White and yellow Rabbit ♂. *Operation:* the L.S.C.G. was cut out.

Examination after operation: lt. pl. the smaller.

„ next day: lt. pl. the larger.

„ after 13 days: lt. pl. the smaller, and the lt. ear artery was no more dilated than normal[1], animal being anæsthetised with A.C.E. mixture, the I.C.B. of both sides were put into 1 per cent. osmic acid as quickly as possible.

Post-mortem examination with methylene blue proved degeneration, by showing fibres in the C.C.B. of the right and not of the left side.

EXPERIMENT XXI. Rabbit ♀. *Operation:* the L.S.C.G. was cut out.

[1] Compare a paper by Pye Smith on "Excision of the Superior Cervical Ganglion": This *Journal*, VIII. 25. 1887.

Examination after the operation: lt. pl. the smaller.

„ next day: lt. pl. the larger.

„ after 11 days: lt. pl. the smaller: and no dilatation of lt. ear artery beyond the normal.

„ after 56 days: ditto.

„ after 62 days: ditto: animal being anæsthetised with A.C.E. mixture; stimulation of I.C.B. of L.S.C.G. at base of skull had no effect on lt. pl.

Post-mortem examination with methylene blue: no fibres shown in the C.C.B. of the L.S.C.G., but fibres normally shown in the C.C.B. of the R.S.C.G.

Experiment XXII. Rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were cut close to the ganglion and sutured with a single extremely fine catgut suture through the cut end of the branches and through the ganglion; the left vagus was also cut and sutured below the superior laryngeal nerve.

Examination after the operation: lt. pl. the smaller.

„ after 3 days: lt. pl. the smaller.

„ after 125 days: lt. pl. the smaller: the animal being anæsthetised with A.C.E. mixture; dissection showed that no union had taken place owing to suture through the ganglion having caused it to degenerate. (1) Stimulation of I.C.B. at base of skull had no effect on lt. pl., (2) stimulation of lt. vagus above or below suture which had healed well, had no effect on heart or œsophagus.

Post-mortem examination with methylene blue: the C.C.B. of the R.S.C.G. showed fibres normally, while the C.C.B. of the L.S.C.G. showed none.

Experiment XXIII. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. were cut close to ganglion and sutured with a single fine catgut suture through the cut end of the branches and through the ganglion: the left vagus was also cut and sutured below superior laryngeal nerve.

Examination after 2 days: lt. pl. slightly the larger.

„ after 4 days: lt. pl. the smaller.

„ after 143 days: lt. pl. the smaller: animal being anæsthetised with A.C.E. mixture, dissection showed the L.S.C.G. again degenerated, (1) stimulation of the I.C.B. of the L.S.C.G. had no effect on lt. pl., (2) stimulation of lt. vagus above or below suture, which had healed well, had no effect on heart or œsophagus.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of the rt. side, but none shown in the C.C.B. of the lt. side.

Experiment XXIV. White and grey rabbit ♀.

Operation: the I.C.B. of the L.S.C.G. were crushed with a ligature.

Examination after the operation: lt. pl. the smaller.

„ next day: lt. pl. the larger.

„ after 2 days: lt. pl. a little the smaller.

„ after 4 days: lt. pl. the smaller.

„ after 259 days: lt. pl. distinctly the smaller but not unsymmetrical: animal being anæsthetised with A.C.E. mixture, (1) stimulation of L.C.S. low down in neck produced immediate and well-marked dilatation of lt. pl., (2) stimulation of L.C.S., after the ganglion and its I.C.B. down to the base of the skull had been dissected out, had similar effect, (3) stimulation of the I.C.B. of the L.S.C.G. also produced immediate and well marked dilatation of lt. pl., (4) the I.C.B. of the L.S.C.G. showed well marked thickening at point where the nerves were originally crushed.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of the R.S.C.G.: a *very* fair number shown in the C.C.B. of the L.S.C.G. but these for the most part were extremely fine.

EXPERIMENT XXV. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. were crushed; also the left vagus above the superior laryngeal nerve was crushed.

Examination after 36 days: lt. pl. the smaller.

„ after 74 days: ditto.

„ after 140 days: both pupils same size but lt. pl. unsymmetrical.

„ after 231 days: ditto. lt. pl. unsymmetrical and does not react to light as normally as right: animal being anæsthetised with A.C.E. mixture; (1) stimulation of L.C.S. had no effect on lt. pl., (2) stimulation of L.C.S. with L.S.C.G. and its I.C.B. dissected out had no effect on lt. pl., (3) stimulation of L.C.B. of L.S.C.G. had no effect on lt. pl., (4) stimulation of lt. vagus above and below point of crushing had no effect on heart or œsophagus, (5) a well marked swelling shown on the I.C.B. of L.S.C.G. at point where nerve was crushed.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of right side: a few fibres extremely fine, shown in the C.C.B. of left side.

EXPERIMENT XXVI. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. cut close to ganglion, the ends being left as much in situ as possible.

Examination next day: lt. pl. the larger.

„ after 5 days: both pupils the same size.

„ after 10 days: lt. pl. the smaller.

„ after 88 days: ditto.

„ after 116 days: both pupils same size but left of unsymmetrical shape.

Examination after 162 days: both pupils same size but lt. pl. unsymmetrical. Animal being anæsthetised with A.C.E. mixture: (1) stimulation of L.C.S. with strong induction shock twice caused most distinct dilatation of pupil, though the dilatation took place much more slowly than normal. However more often on stimulation, no dilatation could be observed, (2) stimulation of L.C.B. of L.S.C.G. with strong current thrice gave a very slow dilatation of pupil but more often no effect.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of the R.S.C.G. but none in the C.C.B. of the L.S.C.G.: consequently regeneration very doubtful, although there seemed to be complete union of nerve at point of original section.

EXPERIMENT XXVII. White and brown rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were crushed; also the lt. vagus above the superior laryngeal nerve was crushed.

Examination after the operation: lt. pl. the smaller.

„ next day: lt. pl. the larger.

„ after 13 days: lt. pl. the smaller.

„ after 58 days: both pupils the same size but left very unsymmetrical.

„ after 89 days: both pupils same size but lt. very unsymmetrical: animal being anæsthetised with A.C.E. mixture: (1) neither stimulation of the L.C.S. nor stimulation of the I.C.B. of the L.S.C.G. produced any certain dilatation of lt. pl. apart from spontaneous movements of pupil which seemed to occur fairly frequently, (2) stimulation of lt. vagus above or below point of injury produced no effect on heart or œsophagus.

EXPERIMENT XXVIII. Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. crushed: also the left vagus above the superior laryngeal nerve crushed.

Examination after the operation: lt. pl. the smaller.

„ next day: ditto.

„ after 2 days: both pupils same size.

„ after 3 days: lt. pl. the smaller.

„ after 255 days: ditto. This rabbit is still alive and is going to be kept for at least six months more in the hope of obtaining regeneration.

EXPERIMENT XXIX. Rabbit ♀. *Operation:* the I.C.B. of the L.S.C.G. were crushed: also the lt. vagus at level of superior laryngeal nerve was cut.

Examination after the operation: lt. pl. the smaller.

„ next day: lt. pl. much the larger.

„ after 2 days: lt. pl. the larger.

„ after 13 days: lt. pl. the smaller.

„ after 254 days: lt. pl. the smaller. This rabbit is still alive and is being kept in the hope of obtaining regeneration.

EXPERIMENT XXX. Yellow Rabbit ♂. *Operation:* the I.C.B. of the L.S.C.G. were crushed: the lt. vagus at level of the superior laryngeal nerve was also crushed.

Examination after the operation: lt. pl. the smaller.

„ next day: both pupils same size.

„ after 3 days: lt. pl. the smaller.

„ after 80 days: lt. pl. a little the smaller.

„ after 146 days: both pupils same size, but left unsymmetrical.

„ after 238 days: ditto. This animal is alive and is being kept in the hope of obtaining regeneration.

EXPERIMENT XXXI. Rabbit ♂. *Operation:* the blood supply to the L.S.C.G. was interfered with by isolating the ganglion from the surrounding tissue.

Examination after 8 days: animal being anæsthetised with A.C.E. mixture: stimulation of the I.C.B of the L.S.C.G. produced well marked dilatation of the lt. pl.

Post-mortem examination with methylene blue: fibres were shown in the C.C.B. of both sides quite normally.

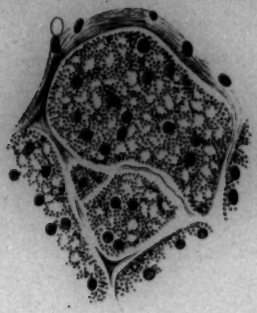
EXPERIMENT XXXII. Rabbit ♂. *Operation:* the L.S.C.G. was isolated from the surrounding tissue and so had its blood supply interfered with: as the left common carotid artery was also ligatured.

Examination after 14 days: animal being anæsthetised with A.C.E. mixture: stimulation of the I.C.B. of the L.S.C.G. caused good dilatation of lt. pl.

Post-mortem examination with methylene blue: fibres shown normally in the C.C.B. of both sides.

Tabular Synopsis of the Records of the Operations and Examinations.

Number of Experiment	OPERATION L.S.C.G. = left superior cervical ganglion I.C.B. = internal carotid branches E.C.B. = external carotid branches	Size of left pupil relatively to right, within two days of operation	Time between operation and final examination	Results of final examination		
				Size of left pupil relatively to right	Are dilatation or vaso-constriction respectively obtainable?	Are fibres shown by methylene blue?
I	I.C.B. of L.S.C.G. cut		4 days	smaller	No	No examination made
II	I.C.B. of L.S.C.G. crushed	larger	5 ,,	,,	No	Ditto.
III	I.C.B. of L.S.C.G. cut		7 ,,	,,	No	Ditto.
IV	I.C.B. of L.S.C.G. cut	equal	27 ,,	,,	No	Ditto.
V	I.C.B. of L.S.C.G. cut		19 hours	,,	Yes	Yes
VI	L.S.C.G. excised		23 ,,	equal	No	Fair number
VII	E.C.B. of L.S.C.G. crushed		23 ,,		Yes	Fair number
VIII	I.C.B. of L.S.C.G. cut		26 ,,		Yes	Fair number
IX	L.S.C.G. excised		30 ,,	smaller	No	A few
X	I.C.B. of L.S.C.G. cut		30½ ,,	equal	No	A few
XI	I.C.B. of L.S.C.G. crushed	larger	41½ ,,	,,	No	No
XII	I.C.B. of L.S.C.G. cut	equal	45 ,,	smaller	No	No
XIII	E.C.B. of L.S.C.G. crushed		48 ,,		No	No
XIV	L.S.C.G. excised		49 ,,	equal	No	No
XV	I.C.B. of L.S.C.G. crushed	equal	52 ,,	smaller	No	No
XVI	I.C.B. of L.S.C.G. cut	equal	3 days	,,	No	No
XVII	E.C.B. of L.S.C.G. crushed		3 ,,		No	No
XVIII	I.C.B. of L.S.C.G. crushed	larger	2 ,,	larger		No
XIX	I.C.B. of R.S.C.G. crushed	equal	3 ,,			No
XX	L.S.C.G. excised	larger	13 ,,	smaller		No
XXI	L.S.C.G. excised	larger	62 ,,	,,	No	No
XXII	I.C.B. of L.S.C.G. cut		125 ,,	,,	No	No
XXIII	I.C.B. of L.S.C.G. cut	larger	143 ,,	,,	No	No
XXIV	I.C.B. of L.S.C.G. crushed	larger	259 ,,	,,	Yes	Yes
XXV	I.C.B. of L.S.C.G. crushed		231 ,,	equal	No	Very few
XXVI	I.C.B. of L.S.C.G. cut	larger	162 ,,	,,	No	No
XXVII	I.C.B. of L.S.C.G. crushed	larger	89 ,,	,,	No	No
XXVIII	I.C.B. of L.S.C.G. crushed	equal	255 ,,	smaller	Rabbit still alive	
XXIX	I.C.B. of L.S.C.G. crushed	much larger	254 ,,	,,	Ditto.	
XXX	I.C.B. of L.S.C.G. crushed	equal	238 ,,	equal	Ditto.	
XXXI	Blood supply of L.S.C.G. disturbed		8 ,,		Yes	Yes
XXXII	Blood supply of L.S.C.G. disturbed		14 ,,		Yes	Yes



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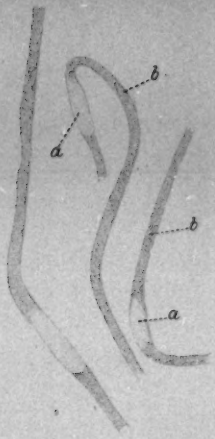

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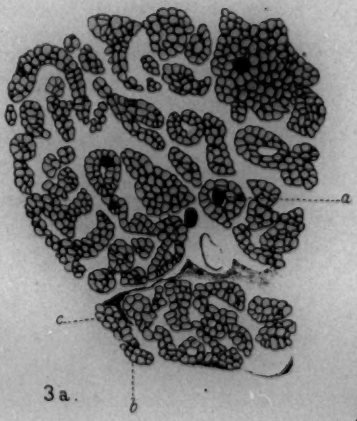

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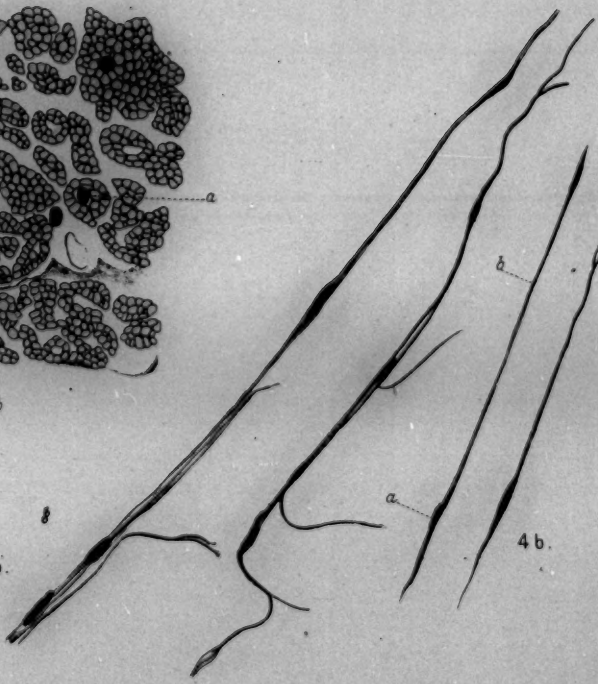
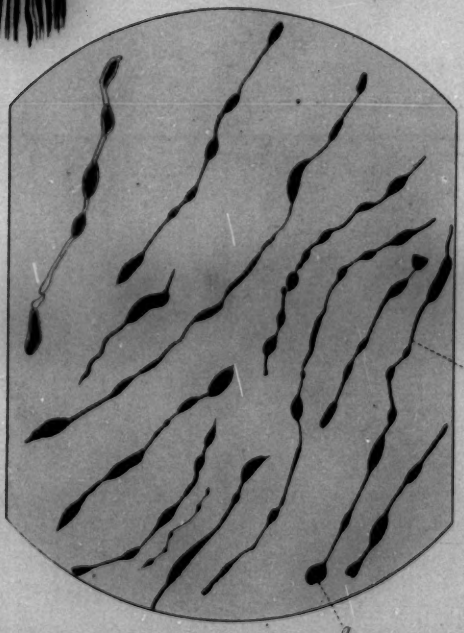


3b.

4b.

4a.



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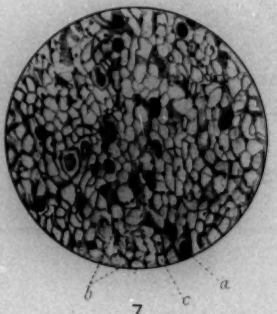

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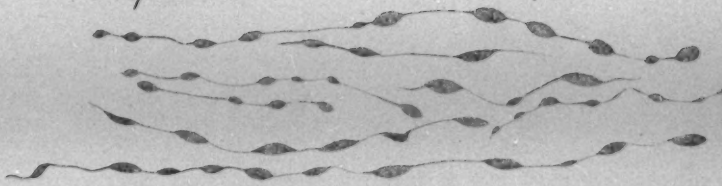

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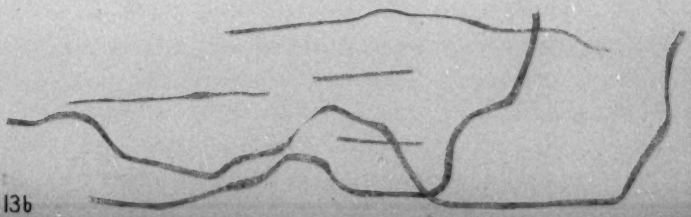

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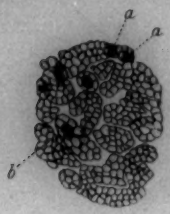

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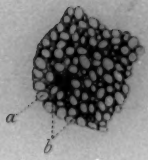

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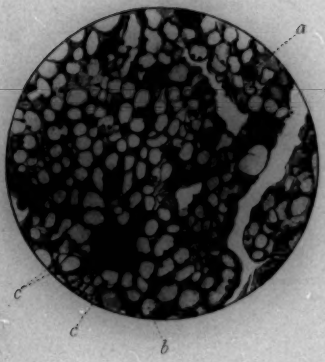

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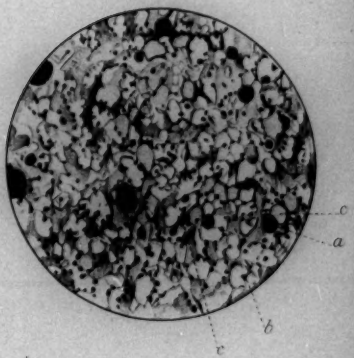

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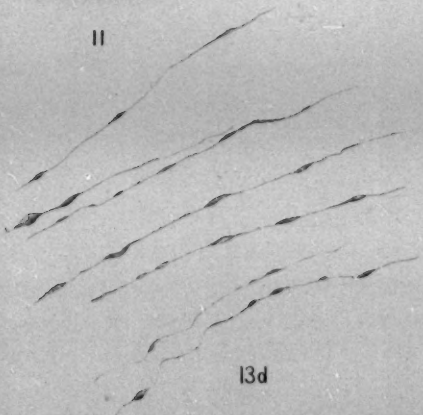

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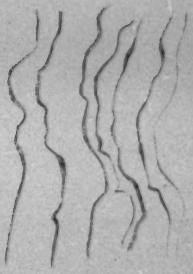

13c

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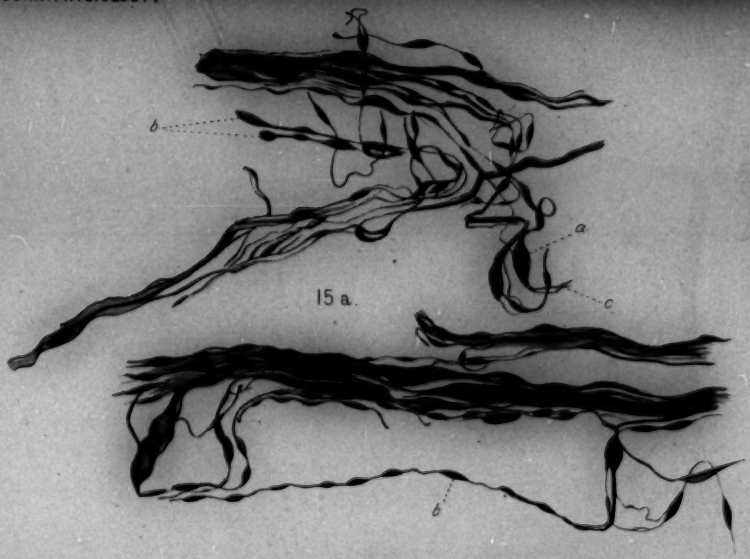
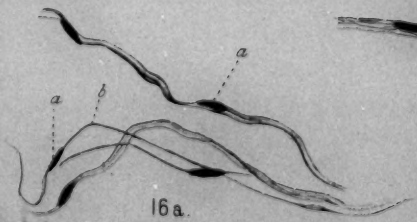


15 a.

14.

15 b.



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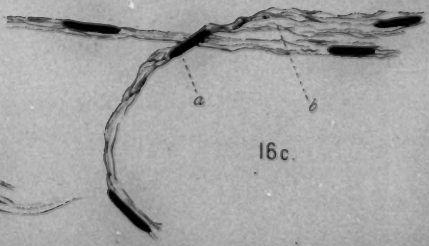

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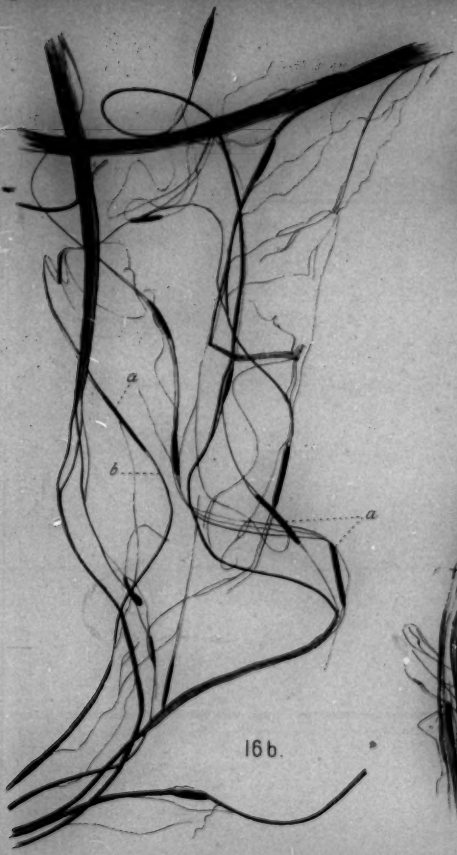

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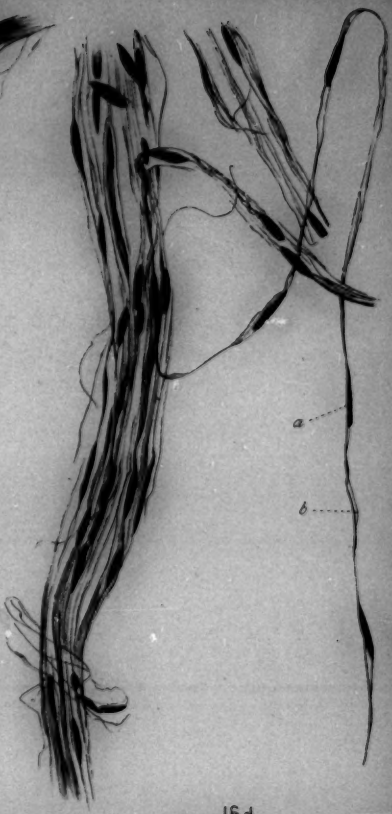
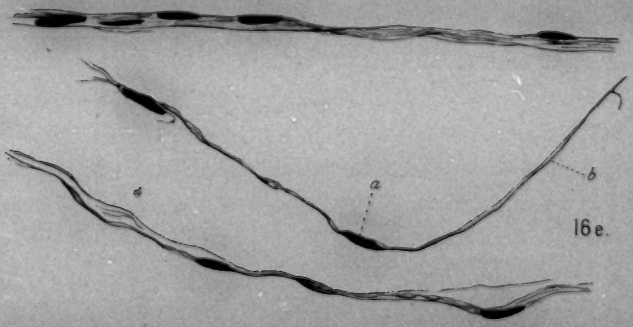


16d.



16e.

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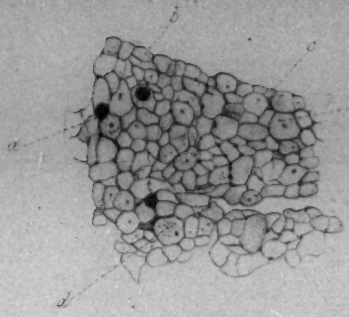
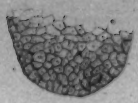


18a.



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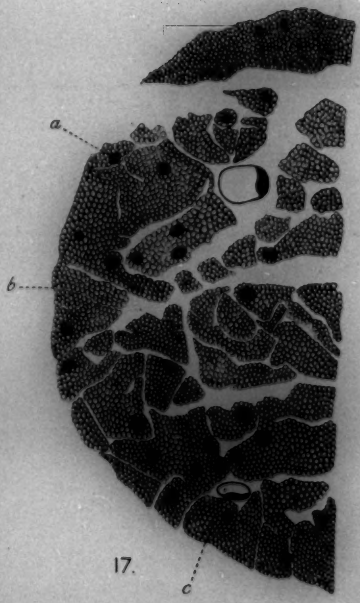

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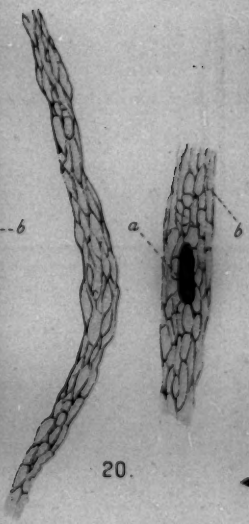
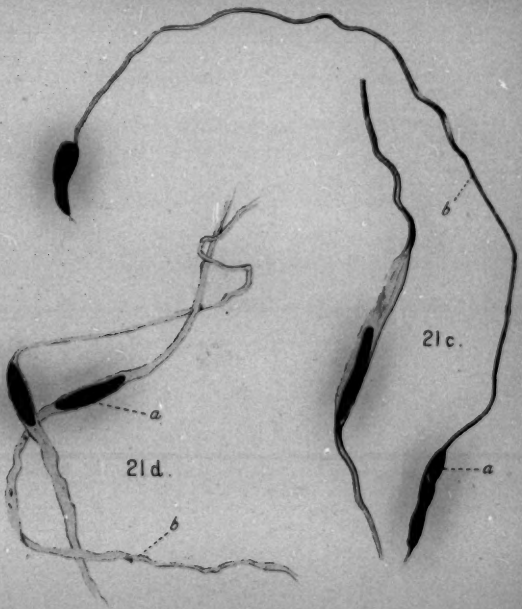
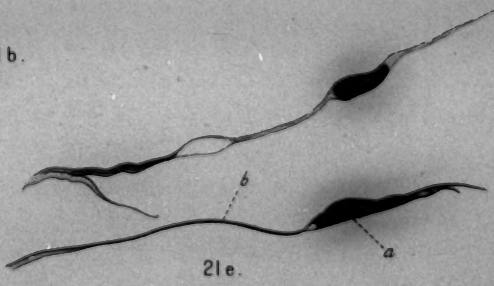


20.

21a.

21b.





21e.

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ROYAL SOCIETY

EXPLANATION OF FIGURES. PLATES I—IV.

Figures 2, 3 a, 3 b, 6, 7, 8, 9, 10, 11, 12, 13 a, 13 b, 14, 17, 18 a, 18 b, 19, 20, 21 a, 21 b, 21 c, 21 d, 21 e are drawn with camera lucida and ocular 4, objective $\frac{1}{18}$ Leitz, magnification 850 diams.

Figures 4 a, 4 b, 5, 13 c, 13 d, 15 a, 15 b, 16 a, 16 b, 16 c, 16 d, 16 e are drawn with camera lucida and ocular 4, objective 7 Leitz, magnification 590 diams.

All the figures with the exception of 18 a and 18 b are reduced by $\frac{1}{3}$ from the original magnification.

The shades of colour in the specimens are represented in the figures as nearly as may be, but it is difficult to get an exact reproduction of the various shades of blue produced by Delafield's hæmatoxylin and chinolin, Grübler's hæmatoxylin, methylene blue.

Plate I.

Fig. 1. Transverse section of the splenic nerve of an ox given by Kölliker, *loc. cit.* p. 35 (strongly magnified).

Fig. 2. Fibres of Remak from the internal carotid branches of the superior cervical ganglion of a rabbit. Teased in iodised serum: (*a*) nucleus, (*b*) core of the fibre inside its sheath.

Fig. 3 a. Transverse section of the splenic nerve of an ox. Osmic acid 1 per cent. Hæmatoxylin chinolin: (*a*) nucleus, (*b*) core of fibre of Remak.

Fig. 3 b. Two fibres of Remak from the vagus of rabbit. To show distinctness of sheath in isolated fibres. 1 per cent. osmic acid. Hæmatoxylin.

Fig. 4 a. Teased preparation of a bundle of fibres of Remak from the carotid canal of a rabbit. Corrosive sublimate. Hæmatoxylin and chinolin: (*a*) nucleus, (*b*) fibre of Remak, (*c*) bundle of fibres.

Fig. 4 b. Isolated fibres of Remak from the same preparation.

Fig. 5. Fibres of Remak from the splenic nerve of an ox. Golgi's method, twice repeated: (*a*) varicosities on fibre, (*b*) shrunken part of fibre.

Plate II.

Fig. 6. Fibres of Remak from the carotid canal of a rabbit: teased in aqueous humour, fixed in 1 per cent. osmic acid. Grübler's hæmatoxylin and eosin in glycerine. These fibres, being somewhat flattened through teasing and crushing, show how the sheath breaks into fibrils: (*a*) nucleus, (*b*) core of fibre, (*c*) sheath.

Fig. 7. Transverse section of splenic nerve of ox. Corrosive sublimate. Hæmatoxylin and chinolin: (*a*) nucleus, (*b*) shrunken part of fibre, (*c*) varicose part of fibre.

Fig. 8. Transverse section of splenic nerve of ox. 2 per cent. ammonium bichromate several months. Hæmatoxylin and chinolin: (*a*) varicose part of fibre, (*b*) shrunken part.

Fig. 9. The outer edge of a transverse section of the splenic nerve of ox. 1 per cent. osmic acid. Hæmatoxylin: (*a*) nucleus indented by core of fibre of Remak, (*b*) fibre of Remak.

Fig. 10. The same section but nearer the centre: (*a*) varicose part of fibre, (*b*) shrunken part of fibre.

Fig. 11. Outer part of a transverse section of the splenic nerve of ox. Osmic acid ·5 per cent. Hæmatoxylin: (*a*) nucleus, (*b*) varicose part of a fibre, (*c*) shrunken part of a fibre.

Fig. 12. The same section but at the centre of it: (*a*) nucleus, (*b*) shrunken part of fibre, (*c*) varicose part of fibre, (*d*) place where some fibres have broken up.

Fig. 13 a. Fibres of Remak from the carotid canal of rabbit. ·06 per cent. methylene blue in ·6 per cent. salt solution.

Fig. 13 b. Similar fibres treated with methylene blue in aqueous humor.

Fig. 13 c. Fibres of Remak from the splenic nerve of ox. ·06 per cent. methylene blue in ·6 per cent. salt solution. Preserved by Bethe's method [*Archiv für mik. Anat.* XLIV. 579. 1895]. Mounted in Canada balsam.

Fig. 13 d. Similar fibres preserved by Bethe's method mounted in glycerine.

Plate III.

Fig. 14. Fibres of Remak from the carotid canal of a rabbit. Teased in aqueous humour. Osmic acid 1 per cent. Grübler's hæmatoxylin eosin (lightly stained). These fibres if anything are rather shrunken: and, not being flattened by the above treatment as in Fig. 6, they do not show a fibril appearance: (*a*) nucleus, (*b*) core of fibre enclosed in sheath.

Fig. 15 a. Teased preparation of splenic nerve of ox. Ammonium bichromate 2 per cent. four months. Hæmatoxylin and chinolin two days: (*a*) nucleus: (*b*) isolated varicose core of a fibre, (*c*) sheath of a fibre.

Fig. 15 b. Isolated varicose cores of fibres from the same preparation.

Fig. 16 a. Two fibres of Remak from the left carotid canal of rabbit, No. IV. After 27 days of degeneration. Corrosive sublimate. Hæmatoxylin and chinolin: (*a*) nucleus, (*b*) sheath.

Fig. 16 b. Fibres of Remak from the left carotid canal of rabbit, No. XXI. After 62 days of degeneration. Treatment as above: (*a*) nucleus, (*b*) shrunken fibre of Remak.

Fig. 16 c. Fibres of Remak from the left carotid canal of rabbit, No. XXVII. After 89 days of degeneration. The sheath, which alone

remains, is very much broken into fibrillæ. Treatment and lettering as above.

Fig. 16 d. Fibres of Remak from the left carotid canal of rabbit, No. XXII. After 125 days of degeneration. Treatment and lettering as above.

Fig. 16 e. Fibres of Remak from the left carotid canal of rabbit, No. XXVI. After 162 days of degeneration. Treatment and lettering as above.

Plate IV.

Fig. 17. Transverse section of the internal carotid branches of the superior cervical ganglion of a rabbit. Osmic acid 1 per cent. Hæmatoxylin: (*a*) nucleus, (*b*) fibre of Remak, (*c*) blood vessel.

Fig. 18 a. Transverse section of the splenic nerve of ox, taken from the centre of the section. Osmic acid ·5 per cent. Kupffer's acid fuchsin method: (*a*) nucleus, (*b*) varicose part of a fibre, (*c*) fibre of Remak a little shrunken, (*d*) granules appearing in core of fibres of Remak.

Fig. 18 b. From the same section but at its edge.

Fig. 19. Bundle of normal fibres of Remak showing a banded appearance. Teased and crushed in aqueous humour. Osmic acid 1 per cent. Grübler's hæmatoxylin eosin: (*a*) nucleus, (*b*) bundle of fibres.

Fig. 20. Bundle of normal fibres of Remak shewing a vacuolar appearance. Treatment and lettering as above.

Fig. 21 a. Normal fibres of Remak from the right carotid canal of rabbit, No. XVIII. Teased and crushed in aqueous humour. Osmic acid 1 per cent. Grübler's hæmatoxylin eosin: (*a*) nucleus, (*b*) core of fibre enclosed in sheath.

Fig. 21 b. Two fibres of Remak from the left carotid canal of rabbit, No. XVIII. After 2 days of degeneration. Treatment as above: (*a*) nucleus, (*b*) fibre of Remak showing discrete granules.

Fig. 21 c. Two fibres of Remak from the left carotid canal of rabbit, No. II. after 5 days of degeneration. Treatment as above: (*a*) nucleus, (*b*) shrunken fibre of Remak.

Fig. 21 d. Two fibres of Remak from the left carotid canal of rabbit, No. XX. After 13 days of degeneration. Treatment and lettering as above.

Fig. 21 e. Two fibres of Remak from the left carotid canal of rabbit, No. XXV. After 231 days of degeneration. Treatment and lettering as above.

ON THE ABSORPTION OF FLUIDS FROM THE CONNECTIVE TISSUE SPACES.

By ERNEST H. STARLING. (Two Figures in Text.)

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Until within the last few years, all workers, who investigated the question of absorption by the blood vessels, confined their experiments to cases in which some substance, not occurring normally in the blood, was introduced into some connective tissue space. That, under these conditions, absorption by the blood vessels does take place, was shown by Majendie, and confirmed in recent years by Ascher[1] as well as by Tubby and myself[2]. Although the ease, with which this interchange by a process of diffusion between blood and extravascular fluids takes place, must be of great importance for the normal metabolism of the tissues (as, *e.g.* the much discussed supply of CaO to the mammary gland-cells), yet such processes will not serve to explain the absorption by the blood vessels of fluids having the same tonicity and the same approximate constitution as the circulating plasma. The fluids contained in the tissue-spaces have the same tonicity and the same composition in salts as blood-plasma. We have to inquire first whether the blood vessels do absorb such isotonic fluids, and secondly the manner in which this absorption takes place.

Evidence as to Absorption by Blood Vessels.

1. *Absorption from the serous cavities.*

A number of experiments have been made recently on the subject of the absorption of isotonic fluids (*e.g.* 1 % salt solution or serum) from the serous cavities. Orlow[3] showed that isotonic fluids were absorbed from the peritoneal cavity with considerable rapidity without producing any corresponding lymph-flow from the thoracic duct, and concluded

[1] *Zeitschrift f. Biologie*, 1893. 247.
[2] This *Journal*, xvi. 140. 1894.
[3] *Pflüger's Archiv*, lix. 170. 1894.

that the fluid was absorbed by means of the blood vessels and that the cells lining the cavity took an active part in the process. His experiments were repeated on the pleura and the peritoneum by Leathes[1] and myself and by Hamburger[2]. We found that 1 % salt solution was taken up fairly rapidly (5 to 10 c.c. an hour) from the pleura. We were unable to decide as to the channels of this absorption; but, from the fact that it went on as usual when a poisonous isotonic solution of sodium fluoride was used, concluded that the absorption must be due to some mechanical or physical condition. Hamburger showed that fluids might be taken up even from the peritoneum of a dead animal. In a recent paper Cohnstein[3], drawing attention to the fact that, after injection of fluid into the peritoneum, we may get a slow slight increase in the lymph-flow from the thoracic duct, concludes that the lymphatics are the sole channels of absorption. I have attempted to decide the question as to the channels of absorption by observing the fate of injected fluids after ligature of the thoracic duct on both sides, combined, in some instances, with ligature of the right innominate vein. Although I have made a number of such experiments in which the fluids were absorbed from the serous cavities, I could never be certain that the pathway by the lymphatics had been blocked by my ligatures. In all cases, carmine which had been injected into the peritoneal cavity some time after the ligature of the ducts, was found in the anterior mediastinal glands, as well as in a gland near the root of the right side of the neck, pointing to the existence of a current of lymph upwards through these glands. I may quote here one of these experiments as an example of the series.

Oct. 11th, 1895. Dog about 10 kilos. Left thoracic duct and right-lymphatic duct ligatured. Right innominate vein ligatured. Then injected 500 c.c. 1 % NaCl solution into peritoneal cavity.

Oct. 12th. Injected 300 c.c. of suspension of carmine in 1 % NaCl solution into peritoneal cavity.

Oct. 14th. Dog killed. All fluid absorbed. No carmine in the distended thoracic duct or in retroperitoneal glands. Carmine present in anterior mediastinal glands and in a gland in neighbourhood of right lymphatic duct.

We must conclude therefore that it is impossible by this method to obtain a definite answer to the question whether blood vessels can absorb isotonic fluids.

[1] This *Journal*, XVIII. 106. 1895.
[2] *Du Bois' Archiv*, 1895. 281.
[3] *Centralblatt f. Physiol.* Sept. 21, 1895.

2. *Effects of artificial anæmia.*

There are however certain facts which have been known to physiologists for the last fifty years and which, to my mind, prove conclusively the absorption by the blood vessels of the fluids in the connective tissue spaces. If an animal be bled to a certain amount, the remaining blood very shortly afterwards is found to be more dilute than before. It contains less hæmoglobin and blood corpuscles and relatively more plasma. The plasma is also more dilute than before, showing that the increase in volume of blood chiefly consists of added water and salts, or at any rate, a fluid which contains less proteid than the plasma. In older works on physiology, we find this dilution of the blood ascribed to an increased flow of lymph from the thoracic duct into the blood. The result of bleeding, however, is to diminish the flow of lymph from the thoracic duct, and the dilution of the blood takes place just as well when the lymph is being led away by a cannula inserted in the duct. As an example of this change I may quote the following figures given by Tscherewkow[1].

(1) Dog 11·4 kilos. Withdrawal of 220 c.c. blood reduced solids of serum from 7·72 % to 7·14 %.

(2) Dog 6·5 kilos. Withdrawal of 150 c.c. blood reduced solids of serum from 7·77 % to 6·47 %.

I have made some experiments to ascertain whether this absorption of fluid is effected only in the abdominal viscera, or whether it may take place in the connective tissues of the peripheral parts of the body.

I find that after *total extirpation* of the abdominal viscera, the organism reacts in the same way as in a normal animal to a considerable bleeding. We may conclude therefore that this absorption of fluid by the blood vessels, after bleeding, takes place throughout the body. This conclusion is also borne out by the experiments of Dr Lazarus Barlow[2] on the specific gravity of the tissues.

3. *Absorption of an artificial œdema.*

In order to put the fact of absorption of isotonic fluids by the blood vessels beyond all doubt, and to exclude all possibility of the concurrence of lymphatics in this process, I have carried out the following experiment.

[1] *Pflüger's Archiv*, LXII. 304. 1895.

[2] *Proc. Physiol. Soc.* 1894. (This *Journal*, XVI. p. xiii.)

The blood of a dog was defibrinated inter vitam, by bleeding, whipping and reinjecting the blood five or six times. [This was to avoid the danger of capillary clots during the subsequent experiment.] The dog was then bled to death, and cannulæ inserted in the femoral arteries and veins on both sides. The right leg was then made œdematous by the injection of 1 % or 1·05 % NaCl solution into the connective tissues by means of a needle. Part of the blood which had been obtained having been set aside for subsequent analysis, the rest was divided into two equal parts. One-half was then led through each limb at a pressure varying between 65 and 85 mm. Hg. By means of an arrangement of tubes and clamps, somewhat similar to that of Ludwig's 'Stromaiche,' it was possible, as soon as all the blood had been led through, to start the circulation afresh from the bottle which had been previously connected with the vein. I was thus carrying on two experiments (one of them being a control) at the same time. Each half of the blood was led through one leg from 12 to 25 times. At the end of this time, the experiment was stopped, and the solids in the whole blood and serum of the three samples of blood estimated, as well as the relative amounts of hæmoglobin in each. It will be seen that the blood, which had been led through the normal leg from 12 to 25 times, was either unaltered, or, as in most cases, underwent slight concentration. The blood which had been led the same number of times through the œdematous leg had in all cases absorbed fluid: both the whole blood and the serum were more dilute and the hæmoglobin percentage was diminished. In these experiments the freezing points of the blood-serum and of the fluid injected to form the œdema, were estimated and care was taken to ensure that the osmotic pressure of the injected fluid was not below that of the blood-serum, so that the absorption of the fluid could not be explained by ordinary osmotic processes. I give here a table showing the results of these experiments.

As the result of these experiments, we may affirm with certainty that isotonic salt solutions can be taken up directly by the blood circulating in the blood vessels.

No. of Exp.	Solids of Blood per cent.			Hæmoglobin (standard = 100)		Solids of serum and freezing points			Composition of œdema fluid and freezing point	No. of times blood transfused through legs
	Standard	From normal leg	From œdematous leg	Blood from normal leg	Blood from œdematous leg	Standard	Normal leg	Œdematous leg		
I	21·00	21·08	19·98	—	—	—	6·6 % Δ = – ·635	6·1 % Δ = – ·635	1 % NaCl Δ = – ·610° C.	12 times
II	18·9	19·2	18·5	—	—	—	—	—	1 % NaCl	16 ,,
III	22·2	22·2	21·4	—	—	—	7·2 % Δ = – ·640	6·7 %	1 % NaCl Δ = – ·610° C.	12 ,,
IV	lost	20·1	19·4	—	—	—	—	—	1·05 % NaCl	12 ,,
V	20·6	(no control)	20·00	—	—	—	—	—	1·05 % NaCl	12 ,,
VI	21·26	21·42	20·56	100	97	—	—	—	1·1 % NaCl	20 ,,
VII	19·97	20·66	19·75	104	96	—	8·28 % Δ = – ·64	7·71 % Δ = – ·64	1·1 % NaCl Δ = – ·660° C.	24 ,,
VIII	20·7	21·2	20·00	103	95	Δ = – ·600	Δ = – ·615	Δ = – ·63	1·03 % NaCl Δ = – ·640° C.	20 ,,
Two Experiments with Serum										
IX	21·12	21·08	21·09	—	—	—	Δ = – ·605	Δ = – ·605	Ox serum Δ = – ·580° C.	15 times
X	19·5	19·9	19·7	102	102	—	Δ = – ·645	Δ = – ·635	Ox serum Δ = – ·585° C.	15 ,,

Mechanism of Absorption by the Blood Vessels.

We have now to consider how this absorption is effected. Are the capillary walls so constituted as to react to a lowering of the capillary pressure with an active absorption of extravascular fluid, *i.e.* is the absorption due to vital activity of the cells? or can we find mechanical conditions that will account for this absorption?

The first possibility that will strike anyone working at the subject is that the absorption, like the transudation, of fluid, may be effected by a process analogous to filtration. Landerer[1] estimated the tissue tension at half to three-quarters of that existing in the capillaries. It is evident that, if such were the case, any considerable fall of intra-capillary pressure would bring it below the tissue-pressure, and a back-filtration into the vessels might occur. Some experiments of Klemensiewicz[2] with regard to the mechanical effects of œdema on the circulation might be quoted against this hypothesis. This latter observer led fluid through a piece of intestine enclosed in an outer tube of glass. He found that at first there was transudation outwards through the intestinal wall and a rise of pressure in the glass tube. As soon however as the pressure in the outer tube reached that obtaining at the venous end of the model capillary, this latter collapsed. Exudation continued however to go on from the arterial end of the capillary. The pressure therefore rose higher and higher in the outer tube until the exuded fluid had caused collapse of the greater part of the intestinal tube. He concluded that a similar sequence of events would take place in œdema and that the exuded fluid would tend to compress the veins, thus raising the pressure in the capillaries still higher and increasing the exudation. A vicious circle was thus established, which only ended with the complete arrest of the circulation through the part affected.

This objection of Klemensiewicz to the possibility of filtration backwards only holds good if the structural relations in the connective tissues are similar to the arrangement of his mechanical model. If however the capillaries, instead of running freely through the connective tissue spaces, are bound to the walls of these spaces by an adventitia of radiating fibres, a rise of pressure in the spaces above that obtaining in

[1] *Die Gewebsspannung in ihrem Einfluss auf die örtliche Blut- und Lymphbewegung.* Leipzig, 1884.

[2] *Sitzb. der k. Akad. der Wissensch.* LXXXIV. 1881 and XCIV. 1886.

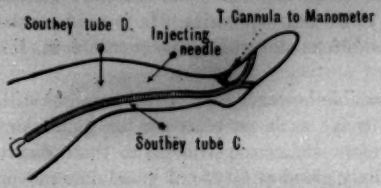
the capillaries will not collapse these latter, but will rather tend to dilate them; and filtration back into the capillary would be structurally possible. If sections be cut of injected œdematous connective tissues, it will be seen that the capillaries are surrounded and supported by such an adventitia of radiating fibres, and have in fact a structure very similar to that figured by Ranvier[1] in the lymphatic gland and by Heidenhain[2] in the section through a villus. In the veins however no such arrangement can be seen, all the fibres surrounding these tubes being apparently disposed concentrically. From a purely anatomical study, it would seem therefore that a filtration back into the capillaries is possible, provided that the rise of pressure in the tissue spaces does not extend to the tissues surrounding the larger veins. It is evident however that the question whether filtration back into the vessels is or is not possible from the connective tissues in most parts of the body, can be only definitely solved by physiological experiment.

I attempted at first to decide this question by observing the capillary circulation in the tongue and web of the frog, while I injected fluid into these parts at a pressure equal to the aortic pressure, which was measured at the same time. In both these localities the production of œdema in this way seemed to leave the capillary circulation unaffected or even to cause a certain quickening. The experiments however were not satisfactory, since it was impossible to ascertain what relation the pressure in the connective tissue spaces bore to the pressure used in injecting the fluid through a fine needle. I therefore devised the following experiment. In a medium-sized dog the blood was first defibrinated intra vitam. A T cannula was then inserted into one of the veins on the dorsum of the foot and connected with a manometer containing normal saline. A cannula was inserted in the internal saphenous vein in the upper part of the thigh and blood allowed to flow away through this; the outflow of blood being measured in graduated cylinders. A needle, connected with a pressure bottle containing warm normal saline, was run into the connective tissue of the leg about one inch in front of the situation of the internal saphenous vein. Two Southey's tubes were also inserted in the connective tissue, one tube (*C*) about 1½ inches from the point of the injecting needle on the other side of the vein, and the other tube (*D*) on the same side of the vein as the injecting needle, but 2 inches nearer the knee. Both these tubes were connected with water manometers. At the same time

[1] *Technisches Lehrbuch der Histologie* (Nicati und Wyss), Fig. 208. 1877.

[2] Cp. *Quain's Anatomy*, 10th Ed. III. Pt. 4. Fig. 110.

the arterial pressure was taken by means of a mercurial manometer connected with the femoral artery of the other leg. The arrangement of the needles and cannulæ is indicated in the accompanying diagram.



I give here the results of one such experiment.

May 1, 1895. Dog about 7 kilos. Cannulæ arranged as in Diagram.

	Pressures (mm. water).				
Time	Vein of foot	Injecting needle	Southey tube C	Southey tube D	Flow in previous 10 mins.
2.25	—	—	—	—	4·2 c.c.
2.35	122	—	—	—	2·8 „
2.50	118	—	—	—	2·8 (15 mins.)
3.0	108	—	—	—	1·8 c.c.
3.10	103	—	—	—	1·5 „
3.10	Injection of 1 % NaCl into connective tissue begun				
3.20	105	315	—	—	1·3 c.c
3.30	↑	315	—	155	0·8 „
3.40	133	355	145	155	0·4 „
3.50	170	265	157	165	0·4 „
3.50	Injection stopped				
4.0	120	107	103	97	0·5 „
4.15	110	80	80	70	1·8 (15 mins.)
4.15	Injection recommenced				
4.25	183	235	130	155	1·3 c.c.
4.40	220	245	160	180	1·8 (15 mins.)
4.40	Injection stopped				
4.50	160	110	110	102	1·3 c.c.
5.0	145	93	97	88	1·6 „
5.10	137	80	95	75	1·8 „
5.20	132	76	87	68	2 „
5.30	127	70	85	67	2·4 „

It will be seen that, with the rise of pressure in the connective tissues, the pressure in the veins of the foot rose, while the outflow from the cannula in the saphenous vein fell, showing conclusively that the effect of a rise of pressure in the connective tissue spaces of the leg is to cause collapse of the big veins and therefore increased pressure in the peripheral veins and capillaries. It is evident therefore that, in the leg, the conditions are analogous to those in Klemensiewicz' experiment.

It seemed possible however that filtration might still occur in other tissues of the body, such as muscular or glandular structures. I carried out therefore similar experiments to those described on the leg, on the submaxillary gland as a type of glandular, and on the tongue as a type of muscular structure. Although these experiments are not so complete as those on the leg, owing to the impossibility of measuring the pressure in the veins peripheral to the œdema, I found that the invariable result of the production of an œdema in these parts was to diminish the outflow of blood. In order to be certain in the case of the tongue that the arterial pressure should be constant throughout, I defibrinated the animal, killed it by bleeding, and then led defibrinated blood at a known pressure through the vessels of the tongue. I had previously divided all the nerves going to the tongue in order, as far as possible, to obviate any changes occurring in the calibre of the arteries.

From these experiments we may conclude that an absorption of fluid by the blood vessels by a process of backward filtration is impossible in the connective tissues of the limbs, in muscles, and in all glandular structures which have an analogous build to the submaxillary gland. Theoretically we may say that absorption by filtration is only possible in those regions of the body where a sudden rise in tissue-pressure will not be propagated to the neighbourhood of the larger veins[1]. Of the existence of such regions however we have no experimental evidence[2].

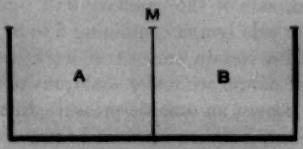
Hamburger has sought to explain absorption of isotonic fluids from the serous cavities by ascribing it to capillary and molecular 'imbibition.' The difficulty in this explanation is the significance which is to be bestowed on the word imbibition. This term for instance is applied to

[1] This argument does not of course apply to the cranium, where the veins are always patent and where, as Hill has shown, filtration of cerebro-spinal fluid into the veins may take place.

[2] The intestinal villus might possibly afford an example of such a structure.

the absorption of fluids by porous bodies, such as animal charcoal. Now such a process may be concerned in the dispersal of the fluid through the interstices of the tissues surrounding the serous cavities, but it is difficult to see how it would lead to the taking up of the fluid by the circulating blood, nor would it explain the delicate balance which has been shown to exist between the volume of circulating blood and intra-capillary pressure, and the absorption from or transudation into the connective tissue-spaces. On the other hand, molecular imbibition, *e.g.* the process by which gelatin will take up water and salts from proteid solutions, may possibly be of the same nature as the process which I am about to describe and which I think is the predominant factor in the absorption of isotonic fluids by the blood vessels.

I believe the explanation is to be found in a property on which much stress was laid by the older physiologists, and which they termed the high endosmotic equivalent of albumen. It must be remembered that the earlier workers used animal membranes in their experiments on osmotic interchanges. These membranes permit the passage of water and salts, but hinder the passage of coagulable proteid. The application of semi-permeable membranes by Pfeffer to the measurement of osmotic pressures, showed that the osmotic pressures of salts and other crystalloids are enormously higher than those of such substances as albumen, and it has therefore been supposed that the osmotic pressure of the proteids in serum being so insignificant must be of no account in physiological processes. The reverse is however the case. Whereas the enormous pressures of the salts and crystalloids in the various fluids of the body are of very little importance for the function of absorption by the blood vessels, the comparatively insignificant osmotic pressure of the albumens is I believe of great importance, and for this reason.



Supposing we have two vessels *A* and *B*, containing salt solutions of different strength, separated by a membrane *M*, which is easily permeable to water and salts. If we imagine that *A* is the more concentrated, there will be a passage of water from *B* to *A*, and the force with which

this attraction of water is effected will be equal to the difference between the osmotic pressures on both sides of the membrane. Now since the membrane is freely permeable to salt, there will also be a passage of salt from *A* to *B*. The layer of fluid in immediate contact with the *A* side of *M* will be therefore less concentrated than *A*, while that in immediate contact with the *B* side of *M* will be more concentrated than *B*; that is to say, the fact that diffusion can take place readily through the membrane minimises the effect of the original difference of osmotic pressures on the transference of fluid. The fluids on the two sides will in time attain equal concentrations; the amount of fluid, *i.e.* water, transferred in the process, diminishing as the diffusibility of the salt increases. Supposing now we add to *A* some substance to which the membrane is impermeable, which we may denote *X*. Since this cannot pass through the membrane, it will exert an osmotic attraction on the water in *B*. Water will flow from *B* to *A* and *B* will become slightly more concentrated. We have now two opposing osmotic forces: the osmotic pressure of *X* in *A* and the osmotic pressure of the excess of salt in *B*. This would tend to produce a position of equilibrium were it not that the condition of excess of salt on one side of a membrane which is permeable to it is an unstable one. The excess of salt must diffuse into *A* and we get a re-establishment of the osmotic difference determined by the presence of *X* in *A*. The final result therefore must be that *A* absorbs the whole of *B*.

Now this illustration represents to some extent conditions existing in the living body. In the limbs and connective tissues generally of the peripheral parts of the body, we have capillaries which are more or less impervious to proteids. As the blood passes under pressure through these capillaries, a certain amount of lymph is filtered through their walls, but in the process it loses the greater part of its proteids. We have therefore on one side of the capillary wall blood-plasma with 8 % proteids, on the other side lymph containing 2 to 3 % proteids. In this separation of proteid a certain amount of work must have been done, and if the proteids of serum are really analogous to the substance *X* of my illustration and possess an osmotic pressure, there must be a difference of osmotic pressure between intra- and extravascular fluids tending to a reabsorption of the latter. It becomes desirable to inquire whether the proteids of serum have any osmotic pressure and if so, what is the extent of this pressure.

To decide these points I have attempted to measure the osmotic pressure of the proteids in the serum directly. The osmometer consists

of a small glass bell provided near the top with two vertical tubulures. Over the mouth of the bell is tied a peritoneal membrane similar to that used by Dr Lazarus Barlow. This was however rendered absolutely watertight by soaking it for some minutes, after it had been tied on, in a 10 % solution of gelatin. The membrane is prevented from bulging by fixing over it a perforated silver or copper plate. The wider of the two tubulures is used for filling the bell with serum. The other one is connected either with a long narrow tube, or with a small mercurial manometer. Three or four of these osmometers are fixed in a wooden disc and arranged to revolve in alternating directions by means of a kitchen jack with their lower ends immersed in salt solution. The salt solution was in most cases 1·03 % NaCl and was therefore slightly hypertonic to the serum.

In all my experiments the fluid in the osmometer began to rise in the tube within two or three hours after the commencement of the experiment, and rose steadily for 3 or 4 days, the final height varying from 30 to 41 mm. Hg. I give here details of two of these experiments.

I. Jan. 27, 1896. Started an osmosis experiment; two osmometers (*C* and *D*) containing serum, while *B* contained a mixture of about $\frac{2}{3}$ serum and $\frac{1}{3}$ outer fluid. Outer fluid = 1 % NaCl. Fluid forced up in the osmometers to heights varying from 3 to 6 inches. A few hours later the fluid in all osmometers had sunk about $\frac{1}{4}$ inch.

Jan. 29. Height of fluid in *C* and *D* = 13 inches = 32·5 cm.
Height of fluid in *B* = 9 inches = 22·5 cm.
Δ of outside fluid at beginning of exp. = −·605° C.
" " at end " = −·615 "
Δ of serum in *C* and *D* at beginning = −·605 "
" " " at end = −·615 "

(The outer fluid, being exposed to the air, concentrated by evaporation during the course of the experiment.)

II. Feb. 4, 1896. Three osmometers filled with serum.

A. Pressure raised to 60 cm. serum. This fell to 53 cm. in 30 mins. and to 50 cm. after 2 hrs.

B. Connected with Hg. manometer. Pressure started at 56 min.

C. Left open.

Feb. 5. (24 hrs later.) At 11 a.m.
Height of *A* = 53 cm. (serum).
Height of *B* = 40 mm. Hg.
C had overflowed.

Feb. 5. At 4 p.m.

Height of A = 53 cm. serum.

Height of B = 40 mm. Hg.

Height of C = 15 cm. serum. Experiment stopped.

At beginning of experiment Δ of serum = − ·600° C.

" " Δ of outer fluid (1·03 % NaCl) = − ·630° C.

At end of experiment Δ of serum = − ·635° C.

" " Δ of outer fluid = − ·635° C.

The serum contained 7·56 % proteids.

The importance of these measurements lies in the fact that, although the osmotic pressure of the proteids of the plasma is so insignificant, it is of an order of magnitude comparable to that of the capillary pressures; and whereas capillary pressure determines transudation, the osmotic pressure of the proteids of the serum determines absorption. Moreover, if we leave the frictional resistance of the capillary wall to the passage of fluid through it out of account, the osmotic attraction of the serum for the extravascular fluid will be proportional to the force expended in the production of this latter, so that, at any given time, there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure there must be increased transudation, until equilibrium is established at a somewhat higher point, when there is a more dilute fluid in the tissue-spaces and therefore a higher absorbing force to balance the increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; and the difference between its (proteid) osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure.

Here then we have the balance of forces necessary to explain the accurate and speedy regulation of the quantity of circulating fluid. It is evident however that we cannot explain in this way the absorption of serum or other fluids rich in proteids from the serous cavities and connective tissues. I would point out however that we have as yet no sufficient evidence that such fluids are absorbed by the blood vessels. If we inject serum into the pleural cavity we find that it is absorbed very much more slowly than is a similar amount of salt solution. The absorption is indeed so slow that it is impossible to exclude the possibility that the whole of it has taken place through the lymphatics.

In two experiments in which I made the limb œdematous with serum instead of salt solution, I could obtain no evidence of absorption of the œdema fluid by the blood vessels. Details of these experiments are given at the end of the table on page 316. The extreme slowness with which inflammatory exudations, *i.e.* fluids rich in proteids, are absorbed in man, also tells against the absorption of these fluids being effected directly by the blood vessels. It is evident however that, if serum be diffused through the meshes of a tissue, the cells of the tissue will feed on the proteids of the serum. As the serum becomes in this way more dilute, its water and diffusible constituents may be taken up by the blood vessels. So far as I know there is no physical process other than filtration by which the absorption of proteids by the blood vessels could be effected. Filtration is excluded by my experiments in the case of the connective tissues of the limbs and peripheral parts of the body, so that a proof that proteids are absorbed in these situations would point to an active intervention of the endothelial cells in the process. All the physiological facts however which are so far definitely ascertained can be explained by the physical processes mentioned in this paper.

Conclusions.

1. Salt solutions, isotonic with the blood-plasma, can be and are absorbed directly by the blood vessels. This statement probably holds good for dropsical fluids containing small percentages of proteids.

2. A backward filtration into the vessels is mechanically impossible in the connective tissues of the limbs, of the muscles and of the glands similar in structure to the submaxillary.

3. The proteids of serum have an osmotic pressure of about 30 mm. to 40 mm. Hg. Absorption of isotonic salt solutions by the blood vessels is determined by this osmotic pressure of the serum proteids. The same factor is probably responsible for the absorption from the tissues which ensues on any general lowering of capillary pressures, *e.g.* artificial anæmia.

4. The proteids of the tissue fluids, when not used up in the tissues themselves, are probably absorbed mainly, if not exclusively, by the lymphatic system.

Note. It is evident that if we translate the term "molecular imbibition" of Hamburger by osmotic pressure of the proteids of the tissues and blood, the explanation of absorption given in the above paper is practically identical with that proposed by this observer.

I must regret the more therefore that in Hamburger's latest contribution to the subject[1], the question is obscured by a mechanical illustration which can have little or no significance for the process of absorption by the blood vessels. In this experiment he has two concentric tubes, the outer one of glass, the inner one of gelatin in the meshes of a nickel gauze cylinder. On filling the outer tube with serum and leading serum through the inner tube, he notices that the outer fluid becomes more concentrated, and that fluid is taken up by the serum passing along the inner tube. Now, on referring to the conditions necessary for the success of the experiment, one sees at once that the pressure in the inner tube must be lower than that in the outer tube, so that Hamburger is really in this experiment imitating transudation rather than absorption. As I have shown above, an absorption, which depends on the extravascular pressure being higher than the intravascular pressure, is mechanically impossible in the case of most of the connective tissues of the body.

[1] *Du Bois' Archiv*, 1896. 36.

ON THE RELATION OF THE OTOCYSTS TO EQUILIBRIUM PHENOMENA IN GALASIMUS PUGILATOR AND PLATYONICHUS OCELLATUS.

By GAYLORD P. CLARK, M.D., *Professor of Physiology in the College of Medicine, Syracuse University, U.S.A.* (Five Figures in Text.)

THE study of equilibrium phenomena in the Crustacea has been mainly directed to those forms the otocysts of which contain otoliths.

Some of the earliest work is that of Delage (1887). His observations were made upon the Schizopoda (Mysis), and the Decapoda-Macrura (Palæmon, Gebia), all otolith-bearing forms. In *Mysis*, which has the otolithic structure in the inner lamella of the tail, he found that removal of the otocysts alone produced no abnormal swimming movements, but when the eyes were also removed there followed repeated rotations around the longitudinal axis of the body when the animal did not rest on a solid support. In *Palæmon*, which has the otocyst in the basal joint of the inner antenna, he found that removal of the otocysts alone was followed by no disturbance of movement, and that destruction of otocysts and eyes caused turning movements and frequent turning upon the back; further, that destruction of one otocyst was followed by changes which were very temporary and indistinct. In *Gebia*, which is an active swimmer, he observed that extraction of one inner antenna caused the animal to incline a little towards the operated side, and that extraction of both occasioned marked disturbance of equilibrium, the animal being turned under-side up and righting itself with difficulty. Removal of eyes in addition appeared to intensify the result. The work of Delage upon the Decapoda-Brachyura (Corystidæ, Carcinus, Polybius), forms without otoliths in the cysts, will be referred to later.

Kreidl (1893) made some very interesting experiments upon *Palæmon* by causing the animal to replace its otoliths after moulting, with the finest metallic iron, and then observing the movements of the animal when an electro-magnet was brought near it from different

directions. The animal was not simply attracted towards the magnet; in certain positions of the magnet it turned away from it. The movements were such as to indicate a physiological reaction, called out by a change of pressure of the "iron otoliths" upon the supporting hairs when they were subject to the attraction of the magnet. Kreidl also reports very prompt reaction of *Palæmon* to rotation, in that the animal runs in the direction opposite to the turning, and when the turning is reversed reverses its direction. When otoliths were removed it did not conduct itself in this way, and if it was blinded in addition it showed absolutely no reaction. When only blinded the reaction was less prompt than in the normal animal. He further found that removal of both otocysts with a fine needle caused no distinct disturbance of equilibrium, while blinding at the same time caused complete lack of orientation, the animal rolling over and lying upon its back, and again, turning several times around its long *axis*. He thus confirms the observations of Delage upon *Palæmon*.

Bunting (1893) studied *Astacus fluviatilis* and found that removal of the otolithic organs caused the animal to become inverted and to swim upon its back. This effect was intensified by loss of the large chelæ, which in this form appear to be a marked mechanical factor in the maintenance of the normal position. Loss of one antennule was without effect. Rotation on the turn-table called forth no compensating movements.

Bethe (1894, 1895) reports that in *Mysis* extirpation of both otoliths is followed by the animal taking the back position in swimming, and that blinding in addition is not needed to produce this disturbance of equilibrium. Extirpation of one cyst caused distinct disturbance, the nature of which the writer does not state, but such as to satisfy him that the otocyst of one side is not capable of acting for both sides. These observations differ from those of Delage upon *Mysis*. In *Astacus fluviatilis* Bethe found that after destruction of the otocysts the animal fell over upon its back, from which position it righted itself with difficulty.

The work thus briefly mentioned furnishes evidence in support of the hypothesis that the otolith in lower and higher animals is an important mechanical factor in the maintenance of equilibrium. By some it is considered a *sine qua non* to the construction of an equilibrium organ. Bethe, in his article "Ueber die Erhaltung des Gleichgewichts," says: "The otolith is foremost in ability to make of the organ an organ of equilibrium. The lack of the otolith in *Carcinus*,

which Hensen specially mentions, appears not to have been known to Delage, otherwise he would not have sought to bring the animal out of its quiet equilibrium by extirpation of the antennules." And in his recent article, "Die Otocyste von Mysis," he remarks: "To-day, where we assume that the otocysts are preeminently an organ for the maintenance of equilibrium, their [the otoliths] importance lies at hand, for only through the stone can the organ serve for orientation in space."

It is noteworthy that very little attention has been given to the Brachyura, the otocysts of which contain no otoliths. Among these forms are active runners and swimmers, and the need of an equilibrium organ would seem to be as great as in those forms which have otoliths. The function of the otolith when present seems to be well determined, but the fact that it is so essential may not be sufficiently established to warrant the assumption that a cyst cannot be an equilibrium organ without it, and therefore that equilibrium experiments upon forms whose cysts are without otoliths are useless. The evident equilibrium function of the semicircular canals in the vertebrates is sufficient to show that an otolith is not a necessity in the construction of an equilibrium organ.

Delage made some observations upon certain of the Brachyura (Corystidæ, Carcinus, Polybius). He reports that in *Corystidæ*, very passive animals, extraction of the inner antennæ is followed by a falling backwards upon the back, and that the animals lie long in that position unless disturbed, then they swim irregularly. In *Carcinus*, a creeper, extraction of the inner antennæ and simultaneous destruction of the eyes were followed by temporary disturbance of equilibrium. In *Polybius*, a swimmer, removal of otocysts was followed by repeated rolling around one or other axis of the body, and difficulty in recovering from the back position. Additional loss of sight intensified the result. Removal of one otocyst was without effect. During the summers of 1894 and 1895 I have made some equilibrium experiments upon two of the Brachyura, in the Marine Biological Laboratory at Woods Holl, Mass. U.S.A.[1] The forms chosen for observation were *Gelasimus pugilator* (Latr.) the "Fiddler Crab," and *Platyonichus ocellatus* (Latr.) the "Lady Crab." These crabs are both very active. *Gelasimus* is a land crab and runs very swiftly upon the tips of its four pairs of ambulatory appendages. The male has one

[1] A preliminary account of the results of the first summer's work appeared in the *Centralblatt für Physiologie*, December 29, 1894.

large chela, developed in some cases upon the right side, in other cases upon the left. This it raises in a defensive attitude, reminding one of the guard arm of a pugilist. *Platyonichus* is a water crab. It moves rapidly over the sand under the water, and by means of the posterior pair of thoracic appendages, which are modified into blades and can execute sculling movements, the crab swims with great ease. By the combined use of its ambulatory and swimming appendages it buries itself with surprising quickness in the sand.

Gelasimus was rotated upon a horizontal turn-table and showed no reaction to slow turning. When fastened and rotated rapidly no after effects were observed. The eye-stalks of *Gelasimus* and *Platyonichus* are long, and project when elevated well beyond the border of the carapace. They were found to show very striking compensating movements when the animals are inclined, and a very careful study was made of these movements in *Gelasimus*. In looking over the literature of the subject since the observations were made the only reference to similar movements in the eye-stalks of the Crustacea which I have found is by Kreidl's paper, in which he remarks, "In this sideward inclination [when the 'iron otoliths' of Palæmon are under the influence of the magnet] the animals execute eye movements, and in such a manner that the eyes, in a turning towards the right around the longitudinal axis of the animal, turn back towards the left." And further on he again remarks, "It is interesting that in these invertebrate animals also, the reflex on the eye-movements is present." I have noted the presence of similar movements in the lobster (*Homarus Americanus*) and cray-fish (*Astacus fluviatilis*), but have studied them only in the two crabs under consideration.

In *Gelasimus* the long moveable stalks when raised project above the front of the carapace at angles of from 30 to 45 degrees with the antero-posterior vertical (sagittal) plane of the animal and therefore with an angle of from 60 to 90 degrees between them. When depressed they lie in furrows, the so-called "orbits," which extend outwards and downwards at angles of 100 degrees with the same plane. For the purpose of observation the crabs were placed on a small eccentric shelf so that the body of the crab would correspond to the centre of a vertical turn-table, and they were held in position by elastic bands passed over the ambulatory appendages at their junction with the body. These bands were of sufficient strength to hold the under part of the body and legs in contact with the surface of the shelf. In order to measure approximately the degree of the compensating movement the vertical

surface of the turn-table back of the crab was divided by lines radiating from the centre into angles of 11¼ degrees. The following results were obtained by observation of many individuals[1].

1. Turning the crab around its antero-posterior axis.

(*a*) Towards its left. This is accompanied by a movement of both eye-stalks around a parallel axis but in the opposite direction. When the turning has extended through an arc of 90 degrees the left stalk has passed into a position either in the sagittal plane of the animal or

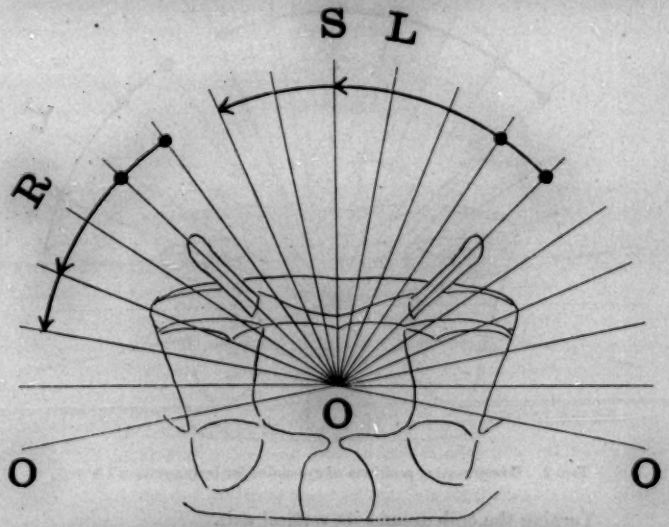


FIG. 1. Compensating positions of eye-stalks for body position 1 *a*.

R right eye-stalk, *L* left eye-stalk.
Dots indicate resting position of eye-stalks.
Arrows indicate direction and extent of the compensating movement.
OOO line of orbits.
SO sagittal plane of body.
Outline of anterior aspect of crab enlarged 9 times.

from 10 to 20 degrees to the right of it, and the right stalk into a position 70 or 80 degrees to the right of it. The angle between the two stalks is then from 45 to 80 degrees.

[1] The nomenclature corresponds to that adopted by Lee in his work on the dog-fish.

(*b*) Turning towards its right. This is accompanied by a movement of both eye-stalks towards the left. The left stalk reaches an angle of 70 to 80 degrees with the sagittal plane, and the right stalk takes a position in this plane or passes to an angle of from 10 to 20 degrees to the left of it.

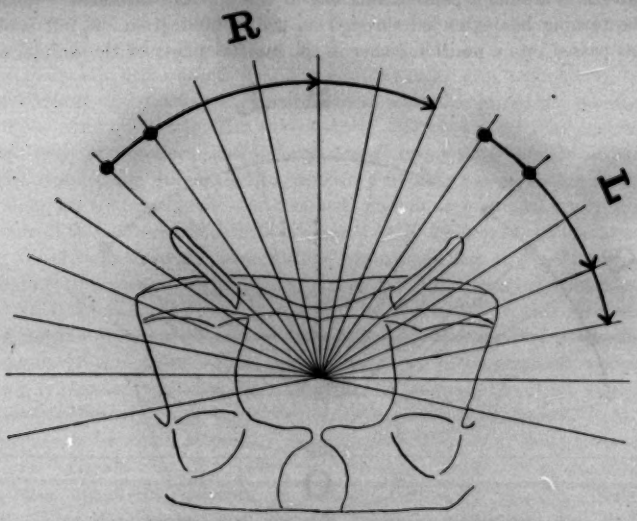


FIG. 2. Compensating positions of eye-stalks for body position 1 *b*.

2. Turning the crab around its vertical axis.

(*a*) Towards its left.

(*b*) Towards its right.

These movements are accompanied by no compensating change of position of the eye-stalks.

When the animal was fastened upon the turn-table both eye-stalks were held at an angle of 10 degrees behind the transverse vertical plane.

3. Turning the crab around its transverse axis.

(*a*) Forwards through an arc of 90 degrees. This was accompanied by a backward inclination of both eye-stalks to an angle of 45 degrees back of the transverse vertical plane.

(*b*) Turning backwards in the opposite direction. This was accompanied by a forward inclination of both eye-stalks to an angle of 20 degrees in front of that plane.

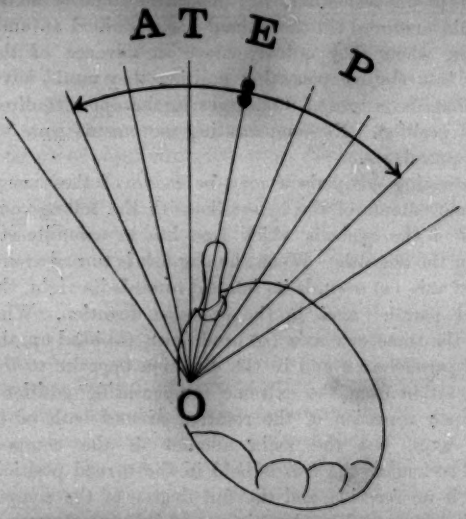


FIG. 3. Compensating positions of eye-stalks for body positions 3 *a*, 3 *b*.
E eye-stalks seen from left of crab.
A anterior, *P* posterior, with reference to crab.
TO transverse plane of body.
Outline of left lateral aspect of crab enlarged 9 times.

Turning the crab in directions intermediate between 1 (*a*) or (*b*) and 3 (*a*) or (*b*), which involve oblique inclinations of the body, causes a combined lateral and antero-posterior bending of the eye-stalks in which the one or the other element may predominate when the plane of inclination tends to approach the lateral or the antero-posterior plane. The compensating movements described are gimbal-like, being sensitive and within certain limits proportional to the amount of turning. The movement ceases before the animal has been turned through the full arc of 90 degrees. I have never seen the stalk which approaches its "orbit" in compensating continue to move until it passed into its groove. When the animal is turned and held in any of the above-mentioned positions, 90 degrees from the normal resting

position, no reaction of the stalks occurs on cessation of the turning, and the stalks remain in their compensating positions so long as the animal is held in the turned position. When the turning around the longitudinal or transverse axes has passed an angle of 90 degrees the stalks remain unmoved till the animal has described an arc of 180 or 200 degrees, when they quickly move in advance of the turning towards or into the compensating position they would have assumed had the animal been turned 90 degrees in the opposite direction from the normal position. No compensating movements were observed in any other appendages.

An interesting comparison may be made of these angular compensating movements of the eye-stalks with the rolling compensating movements of the eyeballs which Lee has so carefully studied and described in the dog-fish. When the dog-fish is turned around (1) the longitudinal axis, (*a*) towards its left, (*b*) towards its right, the eyeballs roll around parallel axes in the opposite direction. When turned around (3) the transverse axis, (*a*) head down, (*b*) head up, the eyeballs roll around parallel axes and in the direction opposite to the turning. A slight reaction from the extreme compensating position has been observed upon cessation of the rotation around both of the above-mentioned axes, but the main amount of the compensation is maintained so long as the fish is held in the turned position. In the crab there is no reaction, and the full degree of the compensation is maintained so long as the animal is out of its normal resting position. When the dog-fish is turned around (2) the vertical axis, (*a*) towards its left, (*b*) towards its right, both eyeballs roll around parallel axes in the opposite direction. When the rotation ceases the reaction is complete, that is, sufficient to carry the eyeballs back to their resting position. In the crab there is no compensation on rotation around the vertical axis. The eye-stalks remain in their resting position whatever the direction or the amount of turning. There are then both in the dog-fish and in the crab compensating eye-movements accompanying rotation in vertical planes, involving inclination of the body, and maintenance of the compensating position, after slight reaction in the dog-fish, with no reaction in the crab. On rotation in the horizontal plane, involving no inclination of the body, compensating eye-movements appear in the fish but not in the crab, and in the fish the reaction is complete.

In *Platyonichus* the compensating eye-stalk movements are very similar to those in *Gelasimus*. When this crab is taken from the

water the eye-stalks are usually kept depressed in the "orbits" so that it was necessary to observe their movements with the animal under water. This was done by fastening the crab to a stick by elastic bands passed over the extended chelæ and then rotating it in an aquarium. No measurement of the degree of movement was made. In comparison with *Gelasimus* the eye-stalks are shorter and more curved. The amount of the lateral movement on rotation around the antero-posterior axis is less, neither stalk passing beyond the sagittal plane of the animal, and the antero-posterior movement is greater, both stalks describing an arc of about 90 degrees when the body is rotated around its transverse axis from a point 90 degrees in front of, to a point 90 degrees back of, the normal resting position. There is no compensation on rotation around the vertical axis. The characteristics of the compensating movements are similar to those described for *Gelasimus*.

The Otocysts.

These structures are situated in the basal segment of the inner antennæ and are formed by an involution of the outside skin. The external opening is closed by a close approximation of its borders. A seam on the external surface of the chitinous shell of the basal segment corresponds with the line of attachment of the cyst to the internal surface. The form of the cyst is very complicated. Hensen describes the cyst of *Carcinus* as typical for the Brachyura. It differs markedly from that of the Macrura. To his description the cysts of the two crabs under consideration essentially correspond. It is of interest to note that Hensen was impressed with a resemblance of the complex Brachyuran otocyst to the labyrinth of the vertebrate ear. Certain views of it reminded him of the semicircular canals, and he describes the cyst as consisting of three half-canals which communicate with each other, besides that part which in forms provided with an otolith contains that structure. No otoliths are present. There extend freely into the water of the cyst a large number of delicate hairs. Some of these, he says, resemble distinctly the ampullar hairs of the vertebrates. Three varieties of these free hairs are described, the hook-hairs (Hakenhaare) of the otolith place, the thread-hairs (Fadenhaare) which project into two of the three half-canals, and the tuft-hairs (Gruppenhaare) crowded together in the outermost corner of the cyst. The tuft-hairs are much coarser and more easily found than the other two forms. In *Gelasimus* there are fifteen of these hairs. They are long, tapering,

smooth and not granular. Hensen states that the other and far finer forms of hairs are also present in this crab, but in my dissections of the sack I have not seen them. In *Platyonichus* the tuft-hairs are more numerous, blunt, smooth and granular. The row of fine thread-hairs is well marked. I have observed no hook-hairs. The general structure of the hairs of the cyst is that of a long hollow shaft with an expanded base, often ball-shape, connected with the chitin wall in such a manner as to leave the hair free to move. The nerves are described as passing from ganglion-cells below the hairs into the hairs, within which they become attached. Bethe in his recent article on *Mysis* describes the nerve-fibres in the caudal cyst of that animal as passing to the very tips of the hair-shafts. Some of Hensen's comments are of interest in connection with the matter under consideration. In speaking of the large number of free-hairs in the otolithless cyst he says, "These, as the case lies, can serve for nothing else than for hearing," and in a footnote, "I would propose to call the process hearing, even if the hairs should bring slower vibrations to perception than our hearing apparatus." Impressed with the auditory function of the otocysts he calls attention to the fact that there may be an auditory apparatus in which the otoliths are lacking, but does not consider it remarkable in view of the fact that a similar condition occurs in the vertebrate labyrinth, and that even in the lobster which has otoliths there are hairs which are never in contact with them. With him the otolith was not a *sine qua non* to the construction of an auditory organ.

Experimental.

Gelasimus pugilator. The otocysts were removed by cutting off the inner antennæ below the basal segment. The crabs were then kept in large glass jars, some of which contained sand kept wet, while others contained only a little sea water. Several of the animals lived long after the operation. Observations were made both immediately after operation and at intervals of several days, so long as the crabs were kept alive. The resting position and movements of the body and the resting position and compensating movements of the elevated eye-stalks were specially noted.

1. *Loss of both inner antennæ.*

(*a*) Effect on body position and locomotion. There was a tendency of the animal to roll over around the transverse axis upon its back when it attempted to run rapidly and upon the tips of its legs. This rolling

was very frequent backwards and occasionally occurred forwards. There was no continuous rolling, either backwards or forwards. When the crab became inverted it usually made immediate efforts to right itself, and sometimes recovered with so much momentum that it rolled over forwards again upon its back. Sometimes it remained a long time motionless on its back. This is not significant, for it is possible to place a normal crab in a similar back position and find a similar hypnotic condition. It was observed that these operated animals soon ceased the attempt to run rapidly and crawled slowly about, with appendages spread so that the body was near the surface on which they were placed. The direction in which they crawled laterally was quite as varied and as often to the right or left as in a normal animal. Placed in water the crabs sink to the bottom and there crawl about. The operated ones in water showed the tendency to roll over around the transverse axis, or a backward and forward rocking movement as if they were uncertain of their equilibrium. A number of cases were kept under observation, some of them for a period of three weeks, and the phenomena above described remained the same. The general condition of the crabs appeared to be unchanged. They burrowed in the sand and fed the same as the normal animals.

(*b*) Effect on position and compensating movements of eye-stalks. No abnormal resting position of the elevated stalks was observed. The stalks remained normally active, being quickly elevated when the crab was placed on its legs, or, if fastened on the turn-table, when its legs were touched, and being as quickly depressed into the "orbits" on tapping the carapace. On rotation in vertical planes the compensating movements of the stalks were found to be markedly diminished (from 45 to 70 degrees), and cases were observed in which they were wholly wanting (see Figs. 4 and 5.)

2. *Loss of one inner antenna.*

(*a*) No abnormal resting position of the body was observed and no constant disturbance of equilibrium on locomotion. In some cases the tendency to roll backwards or forwards was to be seen, sometimes it was temporary, and sometimes persistent.

(*b*) Careful observations were made to determine the effect of one-sided operation upon the position and compensating movements of the eye-stalks, and special attention was given to note whether the stalks were inclined towards the injured side, whether the amount of the compensating movement was less on rotation towards the sound side, and whether the compensating movement of the stalk of the injured

side was less than before the operation. The following table gives the results of these observations. 55 per cent. of the cases showed in the resting position inclination of the stalks towards the injured side. The degree of inclination was small, about 10 degrees; of the above, 50 per cent. were of stalk of sound side, 30 per cent. were of both stalks, 20 per cent. were of stalk of injured side; 94 per cent. of the cases showed less compensation on rotation towards the sound side; of the above, 59 per cent. were of both stalks, 29 per cent. were of stalk of sound side, 12 per cent. were of stalk of injured side; 89 per cent. of the cases showed less compensation of stalk of injured side after than before operation (see Figs. 4 and 5.)

3. *Loss of sight.*

In order to eliminate this sense without injury to the eye-stalks a thick mixture of lamp-black and shellac was carefully applied over the ends of the stalks; after this was done the crabs did not respond to threatening passes made around them, and they collided with objects when creeping. No disturbance of equilibrium was observed. The stalks were elevated and reacted normally to contact stimuli on appendages and carapace. When rotated in vertical planes 50 per cent. showed no diminution of the compensating movements. In the remaining 50 per cent. there were varying degrees of diminished compensation but none extreme. When the sight was eliminated in addition to the loss of both inner antennæ no intensification of the disturbance of equilibrium on locomotion was observed. The compensating eye-movements were completely stopped. The small amount of compensation which remained after removal of both inner antennæ ceased so soon as the sight was eliminated, whether it was done directly after the operation or after an interval of several days; and further, whatever the degree of compensation after the sight alone was eliminated an end was at once put to it by removal of both inner antennæ. If at a later time the black coating was removed from the ends of the eye-stalks the limited amount of compensation was again observed, and again disappeared when the black coating was reapplied. When the eye-stalks were painted in addition to the loss of one inner antenna some compensation was still to be found; and further, when one eye-stalk was painted in addition to loss of both inner antennæ the compensation was not wholly lacking. Compensation would therefore seem to be possible with one intact otocyst and no sight, or with one intact eye and no otocysts.

Figures 4 and 5 are drawn from the positions and compensating

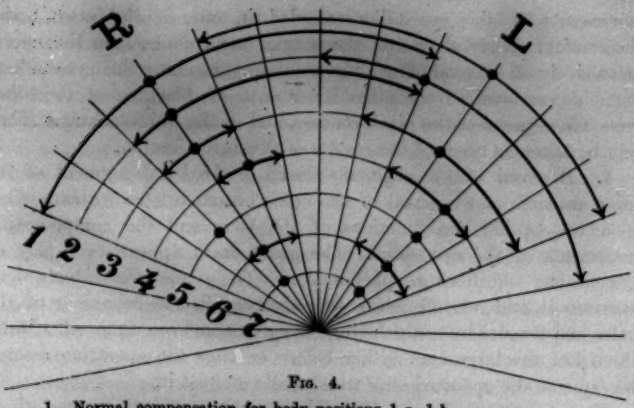


FIG. 4.

1. Normal compensation for body positions 1 *a*, 1 *b*.
2 to 7. Compensation after the following successive operations:
   2. Right inner antenna removed.
   3. Right eye-stalk blacked.
   4. Left inner antenna removed.
   5. Left eye-stalk blacked.
   6. Black coating removed from both eye-stalks.
   7. Both eye-stalks again blacked.

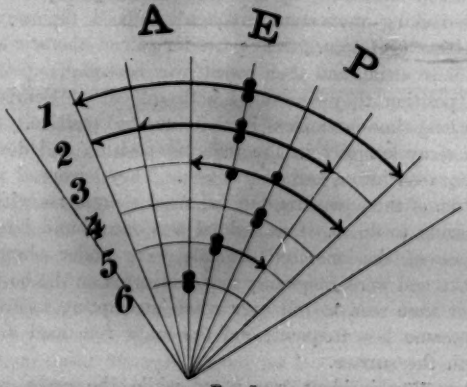


FIG. 5.

1. Normal compensation for body positions 3 *a*, 3 *b*.
2 to 6. Compensation after the following successive operations:
   2. Right inner antenna removed.
   3. Right eye-stalk blacked and left inner antenna removed.
   4. Left eye-stalk blacked.
   5. Black coating removed from both eye-stalks.
   6. Both eye-stalks again blacked.

movements of the eye-stalks recorded in one of the cases under observation. They show first the normal movements, then the effects successively of removal of one inner antenna, blacking the eye-stalk of that side, removal of the other inner antenna, blacking of the other eye-stalk, removal of the black coating, and finally of reblacking. They may be taken as types of the results of this investigation.

4. Removal of the segments distal to the basal segment of the inner antennæ and removal of the outer antennæ were without effect upon the equilibrium functions of the body and the compensating movements of the eye-stalks. The great chela appeared to play no *rôle* in the equilibrium phenomena. The male *Gelasimus* alone possesses it, and no differences were observed in the behaviour of the males and females from which the inner antennæ were removed. Males which lost the large claw either before or after the operation reacted the same as the operated ones which still retained it.

Platyonichus ocellatus. Experiment and observation upon these crabs were essentially the same as upon the form just described. The animals were kept in large aquaria freely supplied with running sea water. Some of them lived many weeks after operation. After removal of both inner antennæ they showed in the water a striking tendency to roll backwards upon the back. They crept about with a to and fro rocking movement, frequently raised themselves into a vertical position as if to use their posterior pair of thoracic appendages to burrow or to swim, and then rolled over backwards upon the back. From this position they recovered with difficulty. Sometimes they remained a long time motionless in that inverted position. Sometimes they swam away laterally in the inverted position and describing an upward curve over into a normal position. They burrowed in the sand on the bottom of the aquarium, but balanced themselves with difficulty when preparing to do so. One animal was once found buried upside down. Some of the operated animals were under observation for several weeks and were frequently found inverted on the bottom of the aquarium or were seen to roll over when attempting to swim. Such attempts became less frequent, and the crabs remained as a rule in contact with the surface.

An interesting incident was noted while the crabs were under observation. I found one morning that one of the crabs had moulted during the night and that in swimming the soft-shelled crab was exhibiting disturbances of equilibrium similar to those observed in animals without the inner antennæ, occasionally rolling over forwards

around its transverse axis. In the course of a few days these abnormal movements gradually ceased. If observations could be made of a number of crabs immediately after moulting it might be found that the equilibrium function of the otocysts is for a time impaired as a result of the process of the separation of the new otocyst from the old one.

The general condition of the operated animals appeared to be unaffected. They seized and devoured pieces of fish when placed in their immediate vicinity but did not seem to appreciate their existence at a distance. No compensating movements of the eye-stalks on rotation in vertical planes were observed at any time in any of the cases from which both inner antennæ had been removed. Eye-stalk movements in response to contact stimuli remained as before operation. After removal of one inner antenna no disturbance of equilibrium was observed. The crabs remained normally active and swam with the same frequency and regularity as before the loss of that structure. The degree of compensation of the eye-stalks was somewhat diminished. Removal of the other inner antenna after an interval of several days produced at once the effects on body and eye-stalk movements characteristic of removal of both inner antennæ together. Elimination of sight by the application of the black mixture produced no abnormal body movements and was followed by no diminution of the compensating movements of the eye-stalks on rotation in vertical planes. Loss of sight in addition to loss of inner antennæ did not increase the disturbance of equilibrium. Without sight and with the loss of one otocyst compensating eye-stalk movements, diminished in degree, were still observed, showing that these coordinated movements may occur on both sides with one intact cyst. The sense of sight appears to play no *rôle* in the compensating eye-stalk movements of *Platyonichus*. The difference observed in *Gelasimus* suggests the question, whether the different media in which these two crabs live may be concerned in the cause of this variation? The loss of other appendages was without effect upon the phenomena under consideration.

A comparison of the results of experimentation on *Gelasimus* and *Platyonichus* with those obtained by other observers on other forms of both Brachyura and Macrura shows, in the main, agreement in the effects of loss of otocysts upon the maintenance of equilibrium. The tendency to assume the back position has been repeatedly noted. In some cases it has appeared only when there was also loss of sight, in other cases the loss of sight has intensified the effect, and in still others the maximum disturbance has followed loss of otocysts alone. Forced

movements are reported in some cases, but I have not observed them in the forms which I have studied. Carrying the comparison up to higher forms Lee and Loeb report that after removal of otoliths from both ears of a dog-fish the animal is frequently found lying and swimming upon its back. A comparison has already been made between the normal compensating eye-stalk movements of these crabs and the normal compensating eyeball movements of the dog-fish. The comparison may now be extended to the effects of loss of the otocysts in the crab and removal of the otoliths in the dog-fish upon these movements. After loss of otocysts in *Gelasimus* the compensation was markedly diminished, and in some cases lacking, in *Platyonichus* it was lacking in all cases. After loss of otoliths of both ears in the dog-fish compensating movements were still present but maintenance of the compensating position on rotation in vertical planes was no longer seen, the reaction being complete on cessation of the rotation, that is, sufficient to restore the eyes to their resting position, a condition that normally appears only on rotation in the horizontal plane. In the crab compensation is reduced to or towards its condition on rotation in the horizontal plane in which it is normally wanting. In the dog-fish the maintenance of compensation is reduced to its condition on rotation in the horizontal plane in which it is normally wanting.

The evidence at hand justifies the view that the coordinate movements of equilibrium phenomena depend in part upon afferent impulses started in certain nerves by the movements of hair cells with which they are connected. Some movement of the hair cell is the essential factor. If an otolith be present its pressure or strain may modify or intensify the movement of the hair cell, but it is conceivable that the movement of the hair cell may occur in the absence of an otolith. A comparative study of the otolithic and related structures from lower to higher forms shows increase of differentiation in both structure and function. There is more differentiation in the Crustacea than in the Ctenophora, and still more in the vertebrates than in the invertebrates. Researches seem to have established quite conclusively the dynamical function of the semicircular canals of the vertebrate ear. The compensating movements in the dog-fish cease when the canals are thrown out of function. The evidence also points to a statical function of the vestibular or otolithic structures. The maintenance of compensation in the dog-fish ceases when the otoliths are removed. But researches do not indicate that the vestibular or otolithic structure is solely statical in function. When it is observed that removal of otoliths from the ears of

a dog-fish may weaken compensation; that stimulation of the otolithic structures may call forth movements of eyes and fins similar to those following stimulation of the semicircular canals, which in turn have been shown to be identical with compensating movements caused by rotation of the normal animal; and that compensating movements of the eyes are present in the crab, and only in those planes in which the compensating movements are normally maintained in the dog-fish, there are seen to be reasons for the view that the hairs of the otolithic structures, or of the cysts which contain no otoliths, may function both dynamically and statically, that they may mediate both the changing and the changed positions of the body in space. The substitution of the terms "Statocyst" and "Statolith" suggested by Verworn for "Otocyst" and "Otolith" seems desirable, but not to the exclusion of the idea that these structures may function in dynamic as well as in static equilibrium. It is a great pleasure to acknowledge my indebtedness to Dr Frederic S. Lee for the assistance he has given to me in this work.

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ON THE NUTRITION OF THE FROG'S HEART.

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THE experiments on which this paper is based were carried out in the same manner and under the same conditions as described in the paper on "A comparison of the relative merits of the perfusion cannulæ of Kronecker and Williams[1]."

My warmest thanks are due to Professor Kronecker, under whose directions the experiments were made.

Whether the heart muscle can or cannot continue working at the expense of its own substance has long been a point of much interest to physiologists. A very large amount of work has been done in the attempt to determine this question, but the results have apparently been such as to divide the workers into two opposite camps—those who are quite convinced that the heart cannot feed on its own substance, and those who hold more or less strongly the opinion that under certain conditions it is capable of nourishing itself, and consequently of working at the expense of its own substance.

Another point of great interest is whether an exhausted heart can be restored to activity by feeding it with fluids other than ones which contain serum albumen, as for example solutions of gum arabic and fluids containing certain salts.

Much of the contradictions in the results obtained as well as in the conclusions drawn, is due either to the adoption of a faulty method, *e.g.* the use of the Williams cannula with which it is almost impossible to fully exhaust the heart and with which, when the heart is in an exhausted or nearly exhausted condition, it is extremely difficult to test the action of a fluid; or else to the faulty application of a method otherwise good, for in many cases the very essential condition of having the heart completely exhausted before testing the supposed nutrient fluid was not complied with.

Before discussing any of these points it is necessary to state clearly what is meant by a fully exhausted frog's heart. It was long ago described by Martius to be one which had been brought first to a standstill by saline solution, and then again exhausted after its temporary resuscitation by an alkaline solution until it gave no contraction to the strongest stimulus. He found that it took on the average from 1½ to 2 hours to produce this condition. In other words, an exhausted heart is one from whose capillary clefts all remnants of blood (nutrient material) have been washed away. No fluid can be considered nutrient unless after feeding a heart exhausted to this complete degree it is capable of restoring the power of contraction.

It may also be as well here to state briefly the opinions arrived at by the various workers, and also where necessary the conditions under which the experiments were made.

Kronecker contends that the heart is incapable of working at the expense of its own substance. He and Stirling[3] state that a heart "can only act equally with the help of continually new nutrient material." The statement that a heart may by means of saline solution be washed to a standstill, and again made to beat by perfusing blood through it, has been confirmed by all subsequent workers.

Kronecker and McGuire[3] and subsequently Handler[4] showed that O_2 was not essential to the maintenance of the heart's contractions.

Merunowicz[5], who was the first to test the action of the salts of the blood on the heart's contractions, showed how solutions of blood-ash strengthen the contractions after they had been reduced by the perfusion of saline solution through the heart.

Stiénon[6] showed the importance of certain salts, especially Na_2CO_3, in sustaining the heart's contractibility.

Gaule[7] found that saline solution rendered alkaline by a little sodium hydrate, made an apparently dead heart beat again for a while. He disagrees with Kronecker's view and inclines to the opinion that the contractions of the heart muscle can be maintained by materials derived from its own substance.

Martius[8] controverts Gaule's view and supports Kronecker's. He points out the great difficulty there is in washing out of the capillary clefts in the walls of the heart all the blood which they contain, and considers that Gaule's hearts, though apparently dead, were not deprived of all their contained blood, but were in a condition of asphyxia, and believes the good effect of the alkali to be due to its action in removing the accumulated CO_2, and thus rendering the

residue of nutrient material available. By washing again with saline solution until the heart is apparently dead, renewing with alkali and repeating the saline, and so continuing, a stage is reached when the alkali has no effect, and the heart refuses absolutely to respond to the strongest stimulus. Then it is that Martius considered his heart to be really exhausted, *i.e.* deprived of all its contained nutrient material. The power to restore such hearts he found was possessed only by such fluids as contained serum albumen (blood, serum, and lymph), while he found glycogen, peptone, syntonin, egg-albumen, casein and myosin to be useless. Finally, he gives expression to the dictum that the heart never works at the cost of its own material.

Von Ott[9] agrees with Kronecker and Martius that the heart cannot contract at the expense of its own substance, and found the contents of the stomach, whey and milk, tested on fully exhausted frogs' hearts, to be nutrient fluids like blood and serum. He further showed that the nutritive action of these fluids depended on the serum albumen which they contained.

Ringer[10] in a series of papers on the action of various salts on the heart finds like Stiénon that in the maintenance of the beats of the heart certain salts are necessary. His investigations have been more systematic and complete than Stiénon's, and he has added much to our knowledge of the precise action of the various saline constituents of the blood, but some of his conclusions are in direct opposition to previously ascertained facts, and by no means warranted by his own results. Thus he states that "the arrest of contractility with saline solution is not due to the removal of pabulum, but that lime and potassium salts are necessary..... and that there is most probably stored up in the muscular tissue material to carry on contractions, and that this material cannot be washed out by a fluid circulating through the cavities of the heart." Martius in his explanation of Gaule's results with the alkaline saline solution had already clearly shown that in some cases the arrest of contractility by saline solution is not due to removal of pabulum, but he showed further that by repeated washing it is possible to remove all traces of stored-up material, and that the alkaline solution then had no effect whatever—only solutions containing albumen being then capable of renewing the heart's activity. Ringer does not appear to have attempted to bring a heart to a condition when it would no longer respond to the action of his own solution. That this is possible, and also that the heart's activity can be restored by blood, is seen on reference to my experiments with

Ringer's solution detailed below. These experiments also dispose of his further statement that "the efficacy of whipped blood plus two parts of salt solution is due to the action of the contained salts."

His claims on behalf of various other substances as possessing nutrient properties similar to serum albumen must be rejected, for the simple reason that they were not tested on fully exhausted hearts. This objection also applies to similar claims made by Howell.

Howell and Cooke[31] also attempt to controvert the statement of Martius that nothing will revive the heart when it has been completely exhausted but serum albumen, and state that they were able to restore a heart "brought into a condition of complete exhaustion according to Martius's view" by feeding it with a purely inorganic diet (Ringer's mixture), and conclude that "a heart is capable of contracting at the expense of its own substance when properly stimulated." This statement is very premature, for Howell also, contrary to previous experience, assumes his hearts to be exhausted when they appeared so after about half-an-hour's perfusion with saline solution. This surely is not "a condition of complete exhaustion according to Martius's view," for Martius took about two hours to wash out the hearts, and then only considered them to be completely washed out or exhausted when they failed to respond to the alkaline mixture and to a strong electrical stimulus.

Heffter[32], who used the Williams apparatus, raises the following amongst other objections to all previous experiments—that the heart was allowed to work much too short a time by the test fluids, and that it was not filled as is the case under normal conditions at each diastole with fresh blood. Now this first objection is really an objection to his own method, and only applies to experiments where the Williams cannula is used. As I have pointed out elsewhere[1] where the heart is approaching exhaustion by means of the Williams cannula, a change made in the perfusing fluid takes a very long time —owing to partial short circuiting—before the first fluid is completely replaced by the test fluid. In similar circumstances the fluid is changed in a few seconds when Kronecker's cannula is used. The latter objection is also fatal in certain circumstances to all work done with the aid of the Williams cannula and for the same reason. Heffter found that *washed* blood corpuscles suspended in a solution of egg-albumen were capable of sustaining the nourishment of the heart, and also that similar corpuscles in a 2 % solution of gum arabic acted splendidly, while such corpuscles in saline solution acted nearly like

saline solution alone: hence, as he agrees with Martius that albumen alone will nourish, he concludes that a nutrient fluid for the frog's heart must not only contain albumen, but it must possess physical properties approximating those of blood.

Albanese[23], working after Heffter, and also using the Williams cannula, thinks that similar results to blood may be obtained by almost any isotonic viscous fluid, and states he found a 2 % solution of gum arabic with a little Na_2CO_3 to be a good nutrient fluid for the frog's heart.

Ohrn[24] working similarly, confirms Albanese's results as regards the solution of gum arabic.

In none of these latter three sets of experiments were the test fluids used on fully exhausted hearts. Indeed I believe it to be almost impossible to fully exhaust a heart by saline solution if the perfusion be attempted through a Williams cannula.

Action of Salts.

That the salts of the blood have a marked influence on the heart's contraction was shown by Merunowicz, and later by Stiénon and Ringer. With the solution introduced by the latter[25] and composed of

100 c.c. of 0·6 % saline solution,
1 c.c. of 1 % solution of bicarb. of soda,
1 c.c. „ „ „ „ chloride of calcium,
0·75 c.c. „ „ „ „ chloride of potassium,

I have made numerous experiments in order to determine precisely the circumstances under which it displays its influence, and to ascertain whether it has any claims to be considered as a nutrient fluid.

This solution, as shown by Ringer himself and also by Howell, is capable of sustaining the action of the frog's heart for hours, and also of restoring a heart which has been apparently exhausted by means of saline solution. It does not however always restore the beats of a heart which has been so exhausted—it however invariably restores the beats in any heart which is not irreparably beyond restoration provided that on stimulation the heart shows the merest trace of a contraction, and also in most cases where the perfusion of saline solution has been continued for some little time after the last faint trace of contraction has disappeared. But only in rare cases where the perfusion has been carried far beyond the latter point. Exactly, however, as Martius found in the case of Gaule's alkaline solution I have found in the

case of Ringer's solution that by alternately washing a heart to apparent exhaustion with the saline solution, and then restoring its beats by Ringer's solution, it can be finally exhausted, so that the Ringer's solution and a strong stimulus produce no contraction. Then on feeding with blood the activity of the completely exhausted heart may again be restored.

The following are two of many examples (the figures indicate the height in mm. of the kymographic tracing)—

I.	Began at 11 a.m.	= 10
	After perfusion of saline for 35 min.	= 0
	„ „ Ringer's solution	= 0
	„ feeding with blood at 12.5 p.m.	= 12
II.	Began at 1.45 p.m.	= 11
	After perfusion of saline for ½ hour	= 0
	„ „ Ringer's solution	= 7
	„ „ Saline solution	= 0
	„ „ Ringer's solution	= 1½
	„ „ Saline solution	= 0
	„ „ Ringer's solution	= 0
	„ feeding with blood at 3.10 p.m.	= 4

Many experiments were made with a view of determining the time which it takes to attain complete exhaustion of the heart by perfusion of Ringer's fluid alone. In these experiments the fluid was constantly perfused through the heart (a large Mariotte bottle being substituted for one of the upright tubes of the frog's heart manometer), with the exception of an occasional short interval of a few seconds, when the perfusion was stopped by the closure of the cocks for the purpose of registering the contractions. In those cases where the heart plethysmograph was used this was not so necessary, and consequently was less frequently done as the contractions were registered during the whole time of perfusion, and so one could see the gradual diminution in the force of the beats. Many of these hearts continued beating for several hours, and the experiments were given up before the desired end was attained. By commencing early in the day, and providing beforehand an enormous quantity of Ringer's fluid, three of the later experiments were successful, and in 5¼, 9¼, and 11¾ hours, the hearts became fully exhausted. Blood succeeded in restoring the beats in the two latter cases.

It is the wonderful sustaining influence of the fluid which compels the consideration of the question as to whether it should be looked

upon as a true nutrient fluid, or in other words, whether it is *per se* capable of restoring and maintaining the heart's beats. However as has been shown when one contrasts its action with a typical and unquestionable nutrient fluid like blood it is found wanting, and even in one condition it is less effective than saline solution. Thus, if when a heart is nearly exhausted perfusion is stopped and it is made to beat under pressure (*i.e.* when by closing the cocks the outflow of the perfusion fluid is prevented, and the heart is connected with the mercurial manometer) with its cavity filled with Ringer's solution the beats are highest at first, and remain high a couple of minutes, but then fall rapidly, as illustrated in the curve.

Fig. 1.

On the other hand, under similar circumstances when the cavity is filled with saline solution the beats are at first small, but they gradually become bigger and bigger, and are sustained at the maximum for a considerable time, and their diminution is as gradual as their rise. Such behaviour is inconsistent with the claims of Ringer's solution to be a true nutrient fluid. Also, as pointed out above, after exhaustion of the ventricle by Ringer's fluid alone, the beats can be restored by feeding with blood; and further, as has also been shown, it has been found possible over and over again where Ringer's solution failed after the heart had been brought to a standstill by saline solution to restore the beats by giving blood. The following curve is an example of this.

Fig. 2.

That part of the tracing to left of *B* shows the negative effect of Ringer's solution, while that to the right of *B* shows the effect of blood.

When Ringer's solution restores the beats, the mere perfusion through the ventricle of a few cc. acts just as well as a large quantity, restoration being almost immediately effected. On the contrary, blood behaves differently, some little time elapses before the effect shows itself, because the blood finds a difficulty in getting into the capillary clefts and can do so only gradually, and so the beats become only by degrees more and more efficient until the maximum is reached. Again, when Ringer's fluid is perfused continuously through the heart there is a gradual diminution in force of the beats, while when blood is used under similar circumstances the force of the beats is uniformly maintained.

Hence it seems pretty clear from these facts that salts alone do not and cannot maintain the heart's contractions—albumen appearing to supply the motive power, while the salts have only a secondary influence. They are of course necessary to the chemical changes which occur during contraction, and may aid in the removal of substances, like lactic acid, formic acid, &c., the accumulation of which may inhibit the heart's activity. These latter substances may be, and most likely are, washed away by the perfusion of the indifferent saline solution. The effect, however, of the alkaline solution not improbably depends, as Martius believed, on its power of removing, by chemical combination, the paralysing CO_2, while possibly the calcium salts serve to render the albuminous residues effective.

The simplest explanation of the various phenomena observed in connection with the use of Ringer's fluid seems to me to be the following. It is only by such a fluid as Ringer's, which contains the salts of the blood in natural proportions, that we can tell when all the nutrient material in the form of blood residue has been removed. When we wash out with an "indifferent" fluid (saline solution) the albuminous material is only very gradually removed, but the salts are easily and consequently very soon completely washed away. Both salts and albumen are necessary to the heart's activity, and are contained in all genuine nutrient fluids (blood, serum, stomach-contents, milk and whey), so when all the former have been removed the beats disappear and the heart appears dead, though albuminous residues still remain. Should Ringer's fluid now be added, it supplies at once the necessary salts, and so we have immediate contractions, which so long as Ringer's fluid is perfusing will continue until the last trace of nutrient material has been washed out of the capillary clefts, and as the reduction in the beats depends now on the removal of the albuminous material, a

removal which is effected very gradually, the heart's action is maintained for a long period. If at any time saline solution is substituted, the beats again rapidly diminish and disappear, for the saline solution has only to remove out of the capillary clefts the traces of the Ringer's fluid.

Gum arabic solution.

The assertions that a 2% solution of gum arabic in normal salt solution is to be considered as a nutrient fluid do not possess much value on account, as already stated, of the method employed.

In order however to test the action of such a fluid I prepared a 2% solution of ordinary commercial gum arabic in saline solution, and made several attempts to restore the beats after the heart had been brought to a standstill by saline solution. Failure occurred in every case. In most instances success followed the subsequent use of Ringer's solution, and in the cases where the latter had no effect the beats were restored by the use of blood. The two following curves serve to illustrate, not only the inutility of the gum solution after the hearts had been rendered almost motionless by the use of saline solution, but also the success of Ringer's solution in restoring the contractions after the gum solution had failed.

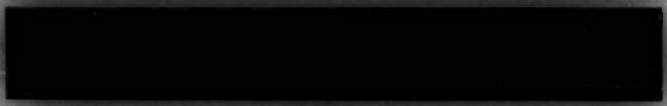

FIG. 3.

The central portion shows the effect of the gum solution after the heart had been almost exhausted by saline solution, while the contractions following the subsequent use of Ringer's solution are indicated on the right.

FIG. 4.

In this case the heart had not been absolutely brought to a standstill by the previous use of the saline solution, and the slight contractions following the use of the gum solution (shown to the left—those following the subsequent use of Ringer's solution being shown to the right) are no greater than the contractions previously registered after the use of the saline solution.

It is possible that by starting and continuing the perfusion of the solution of gum arabic through a fresh heart it may be got to beat for a very lengthened period, as owing to the sticky nature of the solution a layer may form on the internal surface of the ventricle which would prevent, or at least retard, the removal of the nutrient material from the capillary clefts, and so may give rise to an erroneous impression of its nutritive value. This, however, is only conjectural, but at the same time appears to be borne out by some of Heffter's results, who, it must be remembered, *never completely exhausted his hearts.* He found that washed blood-corpuscles suspended in a 2% solution of gum arabic or in a solution of egg-albumen sustained the beats of a heart excellently, while if suspended in saline solution they are no better than saline solution alone. In explanation he says that the nutrient fluid must possess the same physical properties as blood.

The fact that with the two former solutions the beats are well sustained is, I believe, due not to the action of the washed corpuscles, gum, or egg-albumen but to the fact that, in spite of the perfusion, the heart, owing to the consistence of the fluids, retained most if not all of the nutrient material in its tissue[1]; while in the latter case the fluid was more readily capable of washing out the capillary clefts. Certain it is however that when exhaustion was previously brought about by another fluid, *e.g.* saline solution, the perfusion of the solution of gum arabic through the heart had no effect—a result directly contrary to those obtained by Heffter, Albanese and Ohrn. As I merely neutralized my solution and as Heffter and the others appear to have rendered their solutions alkaline with sodium carbonate, it is also quite possible that the apparent good effect was not due to the gum but to the alkali acting on a heart only apparently exhausted. On the other hand, those cases of theirs where the blood appears to act on a partially exhausted heart no better than gum, or where gum fails and blood also fails, are not of much practical value, as they worked with the Williams cannula, and a reference to the discussion of the merits of the cannula will show the possibility of obtaining in such circumstances misleading results. Indeed, judging from my own experience, it seems to be very probable that in many of those cases, as shown for instance in Ohrn's tables, where after the heart had been brought to a stand-still by saline solution negative results were obtained with both blood

[1] In this connection it may be of interest to note that Bowditch in his earlier experiments (Ludwig's *Arbeiten*, 1871, p. 139) successfully used a solution of gum arabic to prevent leakage through the small unligatured heart-pericardial vein.

and gum solution, the latter fluids did not reach the heart at all or only very imperfectly—the conditions being favourable to a short circuiting of the fluid in the Williams cannula.

General Conclusions.

It appears to me to be beyond doubt that the heart is incapable of contracting unless it possesses in its capillary clefts nutrient material (probably serum albumen[1]). In the absence of certain salts (the most important of which are sodium carbonate, calcium and potassium chlorides) this nutrient material cannot be utilized, so that the heart often seems to be exhausted long before such is actually the case. In order to enable it to continue working until the last remnant of blood has been washed out of its substance it is essential that the fluid which is used for perfusion purposes should contain the necessary saline substances. A stage is finally arrived at when the heart is so fully exhausted that no stimulus or no amount of feeding with saline fluids will cause it to contract—that is to say, it cannot contract at the expense of its own substance.

When a heart is in this fully exhausted condition it can only be restored by feeding it with a true nutrient fluid (such as blood, serum, and lymph) containing both serum albumen and salts; the former being most essential, while the latter play a secondary but necessary part in hastening the chemical changes which occur during contraction, as well as neutralizing the effect of such products of metabolism as tend to paralyse the heart's action. No fluid can be pronounced nutrient unless it is capable of revitalizing such an exhausted heart.

A heart is not and cannot be fully washed out in half-an-hour, nor can it with certainty be completely washed out by means of the Williams cannula. The time which it takes to exhaust a heart completely, or in other words to wash out of its capillary clefts the last remaining traces of blood, varies very considerably. The best indication that such a result has been achieved is the failure of the heart to respond to Ringer's fluid. Wash with saline solution to apparent exhaustion, then test with Ringer's fluid, and if it responds, as it probably will, again wash out to apparent exhaustion with the saline solution and once again test with Ringer's fluid, and so on until the latter proves ineffectual and the heart no longer is capable of con-

[1] For at least up to the present no other albuminous body has been proved to be effective.

tracting, no matter how strong a stimulus is applied. If after this a solution is capable of restoring the heart's contractions it may be looked upon with every show of justification as a true nutrient fluid.

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THE ACTION OF CALCIUM, STRONTIUM AND BARIUM SALTS IN PREVENTING COAGULATION OF THE BLOOD.

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It has been shown by Hammarsten[1], Green[2] and Arthus and Pagés[3] that a calcium salt is an essential factor in coagulation of the blood.

Green[2] supposed that the calcium could only act in the form of sulphate and believed that calcium chloride was quite inactive. The work of Ringer and Sainsbury[4], however, has shown that the chloride is almost as effective as the sulphate of calcium.

Arthus and Pagés[3] and Ringer and Sainsbury[4] have demonstrated that the calcium salt may be replaced by a salt of strontium.

Arthus and Pagés[3] believe that barium cannot take the place of calcium in coagulation of the blood. Ringer and Sainsbury[4], on the other hand, have found that barium salts are capable of promoting at least partial coagulation of salted plasma.

A mixture of pure fibrinogen and pure fibrin ferment coagulates only on the addition of a calcium salt. Calcium salts also markedly hasten coagulation when added to salted plasma.

Work upon this subject has so far been entirely confined to demonstrating that calcium is necessary for coagulation of the blood, that it induces coagulation in an otherwise non-coagulable mixture of fibrinogen and ferment and hastens it when added to coagulable mixtures such as salted plasma after dilution.

It is not however with this phase of the action of the salts of calcium upon coagulation of the blood but with another of an entirely opposite nature that I propose to deal in this paper. I have found in fact that,

* This Paper is published without having received the final corrections of the Author. During the passage of the proofs through the Press, Dr Horne fell ill, and after a short illness, died. A promising worker has thus been untimely lost to science. (The Editors.)

when added in certain proportions, the salts of calcium and also of strontium and barium can exercise a well marked retarding or even an entirely preventive influence upon coagulation of the blood.

In my experiments upon this subject I have used the blood of the pig, ox, sheep and rabbit with similar results, and various pure salts, but more especially the chlorides, of calcium, strontium and barium.

The amount of calcium chloride required to delay materially coagulation of the blood is only 0·5 per cent. as illustrated by the following experiment.

Exp. I. Ox blood. To 10 c.c. of a 10 per cent. solution of calcium chloride in distilled water were added 190 c.c. of blood. The mixture contains 0·5 per cent. of $CaCl_2$.

The blood was received direct from the animal into the flask containing the calcium chloride at 4.50 p.m. and well mixed by shaking thoroughly. At 7.30 p.m. the blood could still be poured from one flask into another, but had become slightly thicker. There were also a number of small portions of clot suspended in it. By 10.5 p.m. coagulation was complete.

A control mixture, containing 10 c.c. of distilled water to 190 c.c. of blood from the same animal, coagulated completely in five minutes.

Here then coagulation has been delayed for over three hours by the calcium salt in the proportion of 0·5 per cent. In the proportion of 1 per cent. calcium chloride delays coagulation for a still longer period, as in the next experiment.

Exp. II. Ox blood. To 20 c.c. of a 10 per cent. solution of calcium chloride were added 180 c.c. of blood.

The blood was received into the flask and mixed as before at 4.5 p.m. It was still fluid at 7.50 p.m. on the same evening. At 9.5 a.m. of the following morning there was partial coagulation on the surface and also at the bottom of the flask. Coagulation was almost complete at 5 p.m.

The blood of this animal coagulated normally in a few minutes.

With larger quantities, between 2 and 3 per cent. of the salt, coagulation may be prevented for several days, as in the following experiment.

Exp. III. Ox blood. To 50 c.c. of a 10 per cent. solution of calcium chloride were added 150 c.c. of blood.

The blood and salt solution were mixed at 4.10 p.m. on July 3, and the mixture remained uncoagulated on July 4, 5 and 6. On July 8 at 10 a.m. the blood could still be poured from one flask into another but was thicker from the presence of a number of small clots suspended in it. Putrefaction had commenced. No further change had taken place by 6 p.m. on July 9.

The mixture contains 2·5 per cent. of calcium chloride.

The blood of this animal coagulated normally in a few minutes.

The results obtained with chloride of strontium and chloride of barium are similar to those with the calcium salt and it is therefore unnecessary to give the experiments in detail. One gram of strontium chloride to 200 c.c. of ox blood retarded coagulation for over three hours, and the same amount of barium chloride to 200 c.c. of ox blood retarded coagulation for over seven hours. With larger quantities of these salts the blood may be prevented from coagulating for several days or until putrefaction has set in.

Comparison of the delaying activity of the Chlorides of Calcium, Strontium and Barium upon coagulation of the blood.

It is necessary in making comparisons of the delaying activity of these salts upon coagulation that the solutions of the salts themselves be standardised and that comparative experiments be made on the blood of the same animal and be carried out as far as possible under exactly similar conditions.

A 10 per cent. solution in distilled water was made of each of the chlorides of calcium, strontium and barium and standardised by precipitating the base as oxalate, converting the washed oxalate into oxalic acid and titrating with standard potassium permanganate. It was found that 10·95 c.c. of the calcium chloride, 13·74 c.c. of the strontium chloride, and 13·01 c.c. of the barium chloride solutions, each contained 1 gram of the corresponding salt. It will be sufficiently accurate to take them in the proportion of 11, 14, 13.

With these solutions a large number of comparative experiments were made from which the following may be taken as examples.

Exp. IV. Ox blood.

I.	13 c.c. $BaCl_2$ + 1 c.c. Aq. dest.	+	186 c.c.	blood.
II.	14 c.c. $SrCl_2$	+	„	„
III.	11 c.c. $CaCl_2$ + 3 c.c. „ „	+	„	„
IV.	14 c.c. Aq. dest.	+	„	„

Each of the flasks I., II. and III. contains therefore 1 gram of the corresponding salt.

Mixed at 4.2 p.m. in the above order.

By 4.10 p.m. IV. had coagulated. The other three were still uncoagulated at 10.30 p.m. and at 9.30 a.m. on the following morning. By 8 p.m. of this evening III. showed signs of partial coagulation and was firmly coagulated at 9.30 a.m. next morning, at which time I. and II. were still fluid. I. and II. were still uncoagulated five days after the commencement of the experiment.

Exp. V. Pig's blood.

I. 11 c.c. $CaCl_2$ + 3 c.c. Aq. dest. + 186 c.c. blood.
II. 14 c.c. $SrCl_2$ + „ „
III. 13 c.c. $BaCl_2$ + 1 c.c. „ „ + „ „
IV. 14 c.c. Aq. dest. + „ „

Mixed in the above order at 11.40 a.m.

IV. had coagulated by 11.44 a.m. I. was thicker at 12.55 p.m. and a large part was coagulated at 1.40 p.m. The fluid portion of I. was partially coagulated at 9.15 p.m. and entirely coagulated at 9.30 a.m. next morning. II. and III. were still fluid 32 hours after I. had completely coagulated.

In this experiment the order was reversed for this reason. It was noticed in some of the other experiments that the blood, coming last from an animal, was not so readily prevented from coagulating as that which had been shed first. In the previous experiment it might be said that this was the cause of the calcium blood coagulating before the others. This explanation, however, is negatived by Experiment V.

The reason for the blood coming last from an animal being less readily prevented from coagulating is probably that, as it flows more slowly, the process of coagulation has proceeded to some extent before complete mixture with the salt solution in the flask can be brought about.

In order to obviate this source of error as far as possible a series of comparative experiments were made with calcium and strontium chlorides alone. With two flasks instead of four to manipulate one is better able to obtain each under almost exactly similar circumstances as regards quality of blood, and its thorough admixture with the salt solutions. The results of these experiments are shown in Table I.

Table I.

Comparison of Calcium Chloride with Strontium Chloride.

Exp.	Blood	Percentage of salt	Results	
			Calcium	Strontium
VI	Pig	·5	Coag. in 9 hrs. 35 min.	Fluid after 32 hrs.
VII	„	·5	Coag. after 5 hrs. and within 21 hrs.	Fluid after 57 hrs.
VIII	„	·4	Coag. in 4 hrs. 15 min.	Incomplete coag. in 26 hrs.
IX	„	·4	Coag. in 50 min.	Fluid after 24 hrs.

From this Table it is evident that strontium chloride possesses a

greater power of retarding coagulation of the blood than calcium chloride.

A similar series of experiments were made in order to compare strontium chloride with barium chloride in their action upon coagulation of the blood. The results of some of these are shown in the next Table.

TABLE II.

Comparison of Strontium Chloride with Barium Chloride.

Exp.	Blood	Percentage of salt	Results	
			Strontium	Barium
X	Sheep	·3	Coag. in 9 min.	Fluid after 6 hrs.
XI	„	·3	Coag. in 8 „	„ „
XII	„	·25	Coag. in 4 „	Incomplete coag. in 6 hrs.

The chloride of barium has therefore a much more powerful action in delaying coagulation than the chloride of strontium.

In these experiments I have been comparing the activities of equal weights of the chlorides of calcium, strontium and barium and have found that calcium chloride is the least, and barium chloride the most active. It naturally follows that if the comparison had been based upon their molecular equivalents, perhaps a more correct basis, the results would have brought out in still more striking manner that the relative power of these salts in retarding coagulation of the blood increases in the order calcium, strontium and barium.

Influence of other salts of Calcium, Strontium and Barium upon coagulation of the blood.

Other soluble salts of calcium, strontium and barium are capable of exercising a retarding influence upon coagulation of the blood. Thus for instance calcium nitrate in the proportion of 1 per cent. delayed coagulation in one experiment for over 24 hours. Calcium acetate, in the same proportion, also delayed coagulation but was apparently not so efficacious as the nitrate, coagulation in one experiment being retarded for 15 minutes and in another for 32 minutes. Calcium sulphate and calcium hydrate in saturated watery solutions do not retard coagulation of the blood even when the proportion of solution to blood is large. Strontium nitrate and barium nitrate are both active agents in retarding coagulation. Strontium hydrate delayed coagulation only for a few minutes when added to the extent of 50 c.c. of saturated solution in

water to 150 c.c. of blood. Barium hydrate, on the other hand, in the proportion of 20 c.c. of saturated solution in water to 180 c.c. of blood, delayed coagulation for many hours (in one experiment for several days).

The hydrates of calcium, strontium and barium are almost equally soluble in water, so that there is here another proof that the delaying activity upon coagulation of the blood is greatest with compounds of barium and least with compounds of calcium.

Effect of dilution upon blood in which coagulation has been prevented by the Chlorides of the Calcium group.

In the case of plasma prepared from blood which has been prevented from coagulating by means of oxalate of potassium, dilution with normal saline free from calcium or with distilled water has little or no power to induce coagulation. The influence of the oxalate is not overcome by mere dilution. If, however, blood, to which a calcium or a strontium salt has been added to prevent coagulation, be diluted with distilled water coagulation occurs much more rapidly than in the undiluted blood, and varies in rapidity with the degree of dilution. In this respect therefore it agrees rather with the salted plasmas but differs from these in the comparatively small proportion of the salt required to be added to the blood to retard or prevent coagulation.

A few experiments will suffice to show the effect of dilution upon calcium and strontium blood. In these experiments the blood and diluting fluids were carefully measured from burettes. These experiments were all conducted at the ordinary temperature of the room.

Exp. XIII. Ox blood containing 2·25 per cent. of $CaCl_2$. To each of 8 test tubes were added 5 c.c. of blood. Tubes 1 and 2 kept as controls. To 3, 4, 5, 6, 7, 8 were added 0·5, 1, 2, 3, 4, 5 c.c. respectively of distilled water.

Nos. 8 and 7 coagulated within 14 hours. No. 6 in 17 hrs. 35 min. No. 5 within 38 hours, and No. 4 within 49 hours. At this time there was only partial coagulation in Nos. 3 and 2, and very partial in No. 1.

Exp. XIV. Ox blood containing 1·2 per cent. of $CaCl_2$. Tube 1 contains a mixture of 8 c.c. blood and 2·5 c.c. distilled water, Tube 2 of 5 c.c. blood and 5 c.c. distilled water.

Examined every five minutes. Tube 2 coagulated in 27 minutes and Tube 1 in 72 minutes. The blood itself was still fluid 14 hours afterwards.

Other experiments with calcium blood yielded similar results.

Exp. XV. Pig's blood containing 0·5 per cent. of $SrCl_2$. To each of 4

test tubes were added 5 c.c. of blood. No. 1 kept as control. To Nos. 2, 3, 4 were added 2·5, 5, and 10 c.c. respectively of distilled water.

Examined every 2 minutes. No. 4 coagulated in 10. No. 3 in 14. No. 2 in 21 minutes. No. 1 was still uncoagulated 12 hours after the commencement of the experiment.

Other experiments with strontium blood gave similar results.

Dilution of calcium and strontium blood with distilled water causes therefore a marked acceleration in the process of clotting. The clot which results is firm and complete.

With blood treated with chloride of barium the results are not so definite. There is in this also an acceleration of coagulation upon dilution with distilled water but the resulting clot remains filmy and incomplete. It becomes difficult, therefore, to determine accurately the exact period at which clotting occurred. It is evident, however, from the two following experiments that the accelerating effect of dilution with distilled water upon the coagulation of barium blood is not so marked as in the case of calcium or strontium blood.

Exp. XVI. Ox blood containing 0·5 per cent. of $BaCl_2$. To each of 4 test tubes were added 2·5 c.c. of blood. To Nos. 1, 2, 3, 4, were added 2·5, 5, 10, 15 c.c. respectively of distilled water.

Slight evidence of commencing coagulation in Nos. 3 and 2 in 2 hours 35 minutes, only partial clot in 6 hours, soft clot within 22 hours. In No. 4 slight coagulation in 6 hours, partial clot within 22 hours. No. 1 is still fluid after 22 hours.

Exp. XVII. Pig's blood containing 0·25 per cent. of $BaCl_2$. To each of 4 test tubes were added 5 c.c. of blood. In Nos. 1, 2, 3, 4 were added 3, 5, 10, 15 c.c. respectively of distilled water.

Partial coagulation in Nos. 4 and 3 in 2 hours 45 minutes, and a fairly well marked clot in 5 hours 15 minutes. Slight coagulation in No. 2 in 2 hours 45 minutes, partial clot in 3 hours. No. 1 was still fluid in 5 hours 15 minutes but had undergone partial coagulation within 9 hours.

It need scarcely be stated that this partial coagulation occurring in barium blood upon dilution is not necessarily evidence that barium can replace calcium in coagulation of the blood. The calcium is still present in this blood in its original amount, although perhaps not in the same combination, and may be the cause of the partial clotting which has occurred.

Influence of temperature upon coagulation of blood treated with Calcium, Strontium or Barium Chloride.

All the experiments hitherto described have been carried out at the ordinary temperature of the room (usually about 13° C.). It is known that at temperatures somewhat higher than this, between 30° and 40° C., coagulation, *e.g.* of salted blood plasma and other coagulable fluids, is greatly favoured. The effect of temperature has, therefore, been studied in connection with blood in which coagulation has been prevented in the manner already described. It only requires to be stated that in such blood the effect of a temperature of 30° to 40° C. is also to favour coagulation. An illustrative experiment made upon a specimen of blood treated with calcium chloride may be cited.

Exp. XVIII. Ox blood containing 0·5 per cent. of $CaCl_2$. To each of 3 test tubes were added 10 c.c. of blood. No. 1 kept as control. To No. 2 were added 2·5 c.c., and to No. 3, 5 c.c. of distilled water. Each of these was divided into two portions. Series 1, 2, 3 were kept at the ordinary temperature of the room (10° C. in this case). Series 1 *a*, 2 *a*, 3 *a* were put into a water bath at a temperature of 32° C. The tubes were examined every minute.

No. 3 *a* coagulated in 12 minutes, No. 2 *a* in 18 minutes, No. 3 in 23 minutes, No. 2 in 43 minutes, at which time both Nos. 1 and 1 *a* were still fluid.

Similar results were obtained in experiments upon blood to which strontium or barium chloride had been added. In the case of barium chloride the clot formed at the higher temperature is soft and incomplete like that formed at the lower temperature.

Influence of various salts upon blood treated with Calcium, Strontium or Barium Chloride.

I. *Potassium Oxalate.*

Arthus and Pagés[3] have demonstrated that the addition of potassium oxalate to blood in the proportion of 0·1 per cent. prevents coagulation. Schäfer has recently pointed out, however, that even when the oxalate is added in much larger quantity than 0·1 per cent. a certain degree of clotting occurs in the plasma. This is supposed to be due to the retention in solution of some calcium oxalate from which the calcium necessary for coagulation may be derived. The important

fact for my present purpose is that the retarding or preventive influence of the oxalate upon coagulation of blood is due to the more or less complete precipitation of the calcium as calcium oxalate, and is removed by the addition of a small quantity of calcium or strontium chloride.

The converse is also true. Blood, which has been prevented from coagulating by means of calcium or strontium chloride, is readily induced to coagulate by the addition of a proper proportion of potassium oxalate. The action of the oxalate is here to remove the excess of the calcium or strontium salt. Care has to be taken that excess of oxalate is not added, as thereby the retarding influence of the oxalate itself is brought into play.

In my experiments I have used a strong solution of potassium oxalate, 30 per cent. in distilled water. With a strong solution such as this less oxalate is required to remove the excess of the calcium or strontium chloride and any source of error due to increased rapidity of coagulation from mere dilution is thereby avoided as far as possible. The difference between the rate of coagulation in the control and in the oxalated tubes is also the more marked the less the degree of dilution. The results of some of the experiments with potassium oxalate are shown in Table III. In these experiments the blood and diluting fluids were measured from burettes and the mixtures kept at the ordinary temperature of the room.

TABLE III.

Effect of Potassium Oxalate upon pig's blood containing 1 *per cent. of Calcium Chloride.*

			Results	
Exp.	Blood	Diluting fluid	Aq. dest.	Oxalate
XIX	10 c.c.	0·3 c.c.	7¼ hrs.	22 min.
XX	„	„	7 „	19 „
XXI	„	„	After 6 hrs.	22 „
XXII	„	0·1 c.c.	7 hrs. 9 min.	4 hrs.

Three other experiments with the blood of Experiment XXI. yielded similar results. The time in the above table under the heading "Results" indicates the point when complete coagulation occurred.

In blood to which strontium chloride has been added the oxalate of potassium accelerates coagulation in a similar manner, as shown in the two following experiments.

EXP. XXIII. Pig's blood containing 1 per cent. of $SrCl_2$. To each of 5 test tubes were added 10 c.c. of blood. To No. 1 was added 0·3 c.c. of

distilled water and to each of the others 0·3 c.c. of a 30 per cent. solution of potassium oxalate. All kept at the ordinary temperature. Examined every ten minutes. No. 2 coagulated in 1 hour 42 minutes, Nos. 3, 4, and 5 in 2 hours 22 minutes. No. 1 was still fluid 22 hours after the commencement of the experiment.

Exp. XXIV. Pig's blood containing 1 per cent. of $SrCl_2$. To each of two test tubes were added 25 c.c. of blood. To No. 1 were added 2 c.c. of distilled water, and to No. 2, 0·7 c.c. of the oxalate and 1·3 c.c. of distilled water. Both were kept at the ordinary temperature. No. 2 was coagulated in 3¼ hours. No. 1 was still fluid 48 hours after the commencement of the experiment.

Arthus[3] states that barium chloride, as opposed to chlorides of calcium and strontium, is incapable of inducing coagulation in oxalated blood plasma. My experiments show that the addition of potassium oxalate can remove the inhibitory influence which barium chloride exerts upon coagulation of the blood. The coagulation does not occur so rapidly as in the case of calcium or strontium blood but is sufficiently well marked to leave one in no doubt regarding the result. The clot so produced is usually firm and complete, in contradistinction to the soft and filmy clot obtained by dilution of barium blood. The following experiment illustrates the rate of acceleration.

Exp. XXV. Ox blood containing 0·5 per cent. of $BaCl_2$. To each of 5 test tubes were added 20 c.c. of blood. To No. 1 were added 2 c.c. of distilled water. In Nos. 2, 3, 4, 5 were added 0·1, 0·2, 0·3, 0·4 c.c. respectively of the potassium oxalate (30 per cent.) and 1·9, 1·8, 1·7, 1·6 c.c. respectively of distilled water. All were kept at the ordinary temperature of the room. In No. 5 there was a firm coagulation in 10 hrs. 50 min. and in No. 4 in 18 hrs. 10 min., at which time there was almost complete coagulation in No. 3, partial coagulation in No. 2, while No. 1 was still fluid.

Other experiments with other specimens of blood gave similar results.

II. *Sodium and Potassium Chlorides.*

Ringer has recently strongly insisted on the antagonistic action of sodium and potassium chlorides on the one hand and the chlorides of the calcium group on the other. This antagonism is displayed in their effect upon heart and skeletal muscle and also upon the coagulation of blood and milk. Ringer has found that both sodium chloride and potassium chloride, but especially the former, exercise a restraining influence upon coagulation of the blood which is completely overcome

by the addition of calcium or strontium chloride, and is partially overcome by the addition of barium chloride.

It seemed to me worthy of inquiry to determine whether, conversely, the action of the chlorides of the calcium group in restraining coagulation of the blood could be antagonised by means of sodium or potassium chloride. With this object I have made a series of experiments especially upon blood which has been prevented from coagulating by calcium or strontium chloride. A first experiment was made to determine the effect of a fairly large quantity of sodium chloride.

Exp. XXVI. Pig's blood containing 0·5 per cent. of $CaCl_2$. To tube 1 were added 5 c.c. of blood and 2 c.c. of distilled water, and to tube 2, 5 c.c. of blood and 2 c.c. of an 18 per cent. solution in distilled water of pure sodium chloride. Both were kept at the ordinary temperature of the room. No. 1 coagulated in 8 minutes. No. 2 was still fluid after 29 hours but coagulated within 48 hours.

This experiment shows that the effect of adding fairly large quantities of sodium chloride (in this case to form 5 per cent.) is to materially increase the retardation of coagulation. Much weaker solutions of sodium chloride yield a similar result, as in the following experiment in which 1, 2·25, and 4·5 per cent. solutions in distilled water were used.

Exp. XXVII. Sheep's blood containing 1 per cent. of $CaCl_2$. To each of 4 test tubes were added 5 c.c. of blood. To No. 1 was added 1 c.c. of distilled water, to Nos. 2, 3, 4, 1 c.c. of 1, 2·25 and 4·5 per cent. NaCl respectively. All kept at the ordinary temperature of the room.

No. 1 coagulated in 8 minutes. Nos. 2, 3 were imperfectly coagulated in 3 hours and No. 4 was still fluid. All three were coagulated within 19 hours. In No. 2 the amount of sodium chloride is equal to about 0·16 per cent.

In the subsequent experiments solutions containing 0·3 and 0·5 per cent. of sodium chloride were used. The results of some of these are given in Table IV. In this and subsequent tables the time given under "Results," except when otherwise stated, indicates the point when coagulation occurred. It will save repetition if it be understood that in these and all subsequent experiments the blood and diluting fluids were measured from burettes and the mixtures kept at the ordinary temperature of the room.

TABLE IV.

Action of 0·3 and 0·5 per cent. solutions of Sodium Chloride on Calcium Blood.

All the experiments except two were made with specimens of pig's blood containing 0·5 per cent. of $CaCl_2$. Exps. XXXII. and XXXIII. were made with sheep's blood containing 1 per cent. of $CaCl_2$. 5 c.c. of blood were taken in each case and diluted as shown in the Table.

		Results		
Exp.	Diluting fluid	Aq. dest.	0·3	0·5 per cent. NaCl
XXVIII	1 c.c.	11 min.	16 min.	35 min.
XXIX	1 „	15 „	22 „	35 „
XXX	1 „	15 „	25 „	
XXXI	0·5 „	22 „	68 „	
XXXII	1 „	8 „	30 „	
XXXIII	1 „	23 „	49 „	
XXXIV	0·1 „	43 „		71 min.
XXXV	0·2 „	24 „		38 „
XXXVI	0·3 „	23 „		31 „
XXXVII	0·4 „	20 „		31 „
XXXVIII	0·5 „	14 „		27 „

Similar results were obtained with the addition of fairly large quantities of sodium chloride to blood which had been prevented from coagulating by means of strontium chloride. In the next table are shown the results of three experiments made with 1, 2·25, 4·5, and 9 per cent. solutions of sodium chloride in distilled water upon pig's blood containing 0·5 per cent. of strontium chloride; 5 c.c. of blood were taken in each case and diluted as shown in the Table.

TABLE V.

Action of a large quantity of Sodium Chloride on Strontium Blood.

		Results				
Exp.	Diluting fluid	Aq. dest.	1	2·25	4·5	9 per cent. NaCl
XXXIX	2·5 c.c.	14 min.	35 min.	49 min.	5 hrs. 31 min.	Uncoag. in 24 hrs.
XL	1 „	18 „	70 „	4 hrs.	Uncoag. in 7 hrs.	Uncoag. in 7 „
XLI	1 „	17 „	35 „	45 min.	Uncoag. in 6 „	Uncoag. in 6 „

The effect of a 0·5 per cent. solution of sodium chloride upon the coagulation of blood to which strontium chloride has been added is shown in Table VI. These experiments with one exception were made

with pig's blood containing in the first four 0·5 and in the other two 1 per cent. of strontium chloride. The last experiment was made with sheep's blood containing 0·75 per cent. of strontium chloride. The amount of blood and diluting fluid used in each case are given in the Table.

TABLE VI.

Action of 0·5 per cent. solution of Sodium Chloride on Strontium Blood.

			Results	
Exp.	Blood	Diluting fluid	Aq. dest.	0·5 per cent. NaCl
XLII	5 c.c.	1 c.c.	16 min.	35 min.
XLIII	5 „	0·5 „	18 „	22 „
XLIV	25 „	10 „	31 „	44 „
XLV	50 „	10 „	54 „	72 „
XLVI	25 „	10 „	39 „	59 „
XLVII	25 „	10 „	36 „	After 5 hrs.
XLVIII	25 „	10 „	34 „	1 hr. 21 min.

Potassium chloride acts in a similar manner to sodium chloride upon blood to which calcium or strontium chloride has been added. Thus in one experiment made with pig's blood containing 0·5 per cent. of calcium chloride there were added to 5 c.c. of the blood in each of three test tubes 1 c.c. of distilled water, 1 and 2·25 per cent. solutions of potassium chloride respectively. No. 1 coagulated in 18 min., No. 2 in 25 min. and No. 3 in 34 min. In another experiment to 5 c.c. in each of two test tubes of pig's blood containing 1 per cent. of calcium chloride were added 1 c.c. of distilled water and 1 per cent. solution of potassium chloride respectively. No. 1 coagulated in 59 min., No. 2 in 1 hr. 48 min. The potassium chloride in these experiments has simply manifested its tendency to delay coagulation of the blood. The same point is brought out if weaker solutions of potassium chloride be used as indicated in Table VII. upon calcium blood, and in Table VIII. upon strontium blood.

TABLE VII.

Action of 0·3 and 0·5 per cent. solutions of Potassium Chloride on Calcium Blood.

All of these experiments were made with pig's blood containing 1 per cent. of $CaCl_2$ with the exception of the first, which contains 0·5 per cent. of

$CaCl_2$. 5 c.c. of blood were used in the first three experiments and 25 c.c. in the remainder. The amount of diluting fluid used is given in the Table.

Exp.	Diluting fluid	Results		
		Aq. dest.	0·3	0·5 per cent. KCl
XLIX	0·5 c.c.	35 min.	40 min.	51 min.
L	1 „	59 „	81 „	98 „
LI	1 „	54 „	78 „	78 „
LII	10 „	40 „	54 „	75 „
LIII	5 „	2 hrs. 5 „	3 hrs. 46 „	3 hrs. 46 „
LIV	5 „	2 „ 43 „	After 4 hrs.	After 4 hrs.

TABLE VIII.

Action of 0·5 per cent. solution of Potassium Chloride on Strontium Blood.

The first experiment was made with pig's blood containing 0·5 per cent., the second with pig's blood containing 1 per cent. and the remainder with sheep's blood containing 0·75 per cent. of $SrCl_2$. 25 c.c. were taken in each case and diluted with 10 c.c. of distilled water and 0·5 per cent. KCl respectively.

Exp.	Results	
	Aq. dest.	0·5 per cent. KCl
LV	10 min.	16 min.
LVI	1 hr. 40 min.	After 6 hrs.
LVII	22 min.	42 min.
LVIII	26 „	54 „
LIX	34 „	1 hr. 16 min.
LX	32 „	2 hrs.

Very occasionally a different result is obtained with this amount of potassium chloride on strontium blood, the two tubes either coagulating at the same time or as in one experiment, the potassium chloride tube coagulating before the other. This result may have been accidental and does not affect the general conclusion.

It is evident from the foregoing experiments that in the quantities which have been used neither sodium nor potassium chloride exercises any antagonism to the delaying action of calcium or strontium chloride upon coagulation of the blood. On the contrary we have, in these experiments, another demonstration of the fact that both sodium and potassium chlorides tend to delay coagulation of the blood. When added even in small quantities (*e.g.* to form ·07 per cent.) to blood in

which coagulation has been delayed by means of calcium or strontium chloride, they still further retard the process of coagulation.

No experiments have been made upon the action of these salts upon barium blood. There can be little doubt, however, that what applies to calcium and strontium blood applies also with but slight modifications to barium blood.

Remarks.

The only reference I have been able to discover in the literature of blood coagulation which has a direct bearing on the subject of this research is in a paper by Green. It is there stated that barium chloride can prevent coagulation of the blood when added in sufficient quantity. Believing that the calcium, to play its part in coagulation of blood, must be in the form of calcium sulphate, Green explained the preventive action of the barium chloride in supposing that it converted the active calcium sulphate into the inactive calcium chloride. Apart from the fact, which is now generally accepted, that the calcium is perfectly capable of playing its part in coagulation of the blood in the form of calcium chloride, it is evident from the facts brought out in this research that Green's explanation of the action of barium chloride in preventing coagulation is inadequate. For not only barium and strontium chloride but also the chloride of calcium itself is capable of delaying or preventing coagulation of the blood. I do not propose to discuss in this paper the possible explanations which might be brought forward, and as it is my intention to prosecute this subject further it will be better to postpone the explanation I would advance until it has been submitted to the test of further experiment.

Summary.

The results of this research may be briefly summarised.

1. The soluble salts of calcium, strontium, and barium retard or prevent coagulation of the blood when present in the proportion of 0·5 per cent. and upwards.

2. Barium salts have a more powerful preventive action upon coagulation of the blood than strontium, and strontium than calcium salts. Barium chloride materially retards coagulation of the blood in the proportion of 0·25 per cent.

3. Calcium hydrate in saturated watery solutions does not retard, strontium hydrate retards only slightly, while barium hydrate is highly effective in retarding coagulation of the blood.

4. The delaying influence of calcium, strontium and barium chlorides upon coagulation of the blood is overcome by dilution with distilled water, and by the addition of a suitable quantity of potassium oxalate.

5. Coagulation of blood, treated with calcium, strontium or barium chloride, is accelerated by a temperature of 30° to 40° C.

6. Sodium chloride and potassium chloride, added to the extent of 0·07 per cent. and upwards, do not accelerate but still further retard the coagulation of blood which has been treated with calcium or strontium chloride.

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THE INNERVATION OF THE PELVIC AND ADJOINING VISCERA.

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PART VI. HISTOLOGICAL AND PHYSIOLOGICAL OBSERVATIONS UPON THE EFFECTS OF SECTION OF THE SACRAL NERVES. (One Figure in Text.)

We stated in Part IV. that stimulation of the sacral nerves has no observable effect on the internal generative organs, and from this we concluded that the sacral nerves send no efferent fibres to these organs. It seemed to us imperative to test this conclusion in some other way, and if it were confirmed, it seemed highly desirable to ascertain also whether the internal generative organs are or are not supplied with afferent fibres by the sacral nerves. The obvious way of applying a test, was to cut the sacral nerves and to observe microscopically the course of the degenerating nerve fibres.

We have accordingly made five experiments, as follows:

Ether and morphia were used as anæsthetics; of the latter 2 to 3 c.c. were injected subcutaneously. The usual antiseptic precautions were employed.

Exp. 1. Cat. Female. Cauda equina cut above the 2nd sacral spinal ganglia. This is equivalent to cutting the roots of the 2nd sacral and of the lower nerves centrally of the posterior root ganglion. Thus efferent fibres only will degenerate in the nervus erigens. The animal was killed under anæsthetics at the end of *4 days*. The lumbo-sacral nerves belonged to the posterior (*a*) class.

Exp. 2. Cat. Female. Cauda equina cut above the 2nd sacral spinal ganglia, and the animal killed at the end of *12 days*. The nerves were of posterior (*a*) class.

Exp. 3. Cat. Male. Cauda equina cut above the 3rd sacral spinal ganglia; the 2nd and 3rd sacral nerves on the left side, and the 2nd sacral nerve on the right side, cut peripherally of the posterior root ganglia. Animal killed at the end of *7 days*. The nerves were of the median class.

Exp. 4. Cat. Female. The 1st, 2nd, 3rd sacral and 1st coccygeal

nerves cut peripherally of the posterior root ganglia on the *left* side. Animal killed at the end of *11 days*. The nerves were of the posterior (α) class.

Exp. 5. Rabbit. Female. The 1st, 2nd, 3rd, 4th sacral and 1st coccygeal nerves were cut peripherally of the posterior root ganglia on the *left* side. Animal killed at the end of *7 days*. The nerves were of the median class.

In each case, both physiological and histological observations were made. We will take the latter first, since they were the main object of the experiments.

Histological Observations.

The peripheral nerve fibres were treated as follows:—

The pelvic plexus, with the pelvic nerve (nervus erigens) and hypogastric nerve, was exposed on each side. Any fat lying over the nerve fibres was carefully removed; and the plexus, together with the uterus, vagina, bladder and urethra—or the corresponding organs in the male—was excised, spread out on a plate, and treated for a day with 1 p.c. osmic acid. It was then placed in water, and the numerous branches of the pelvic plexus dissected out. In the intervals of dissection, the preparation was placed in 50 p.c. alcohol. The plexus was carefully drawn, examined under a microscope with a magnifying power of about 100 diameters, and the presence and approximate number of normal and degenerated medullated fibres in each strand noted. When it seemed advisable, a small piece of a nerve strand was cut out, teased, and the number of normal and degenerated medullated fibres counted under a magnification of 300 to 400 diameters.

In Exp. 1 (4 days) the degeneration was as a rule not sufficiently advanced to distinguish between fibres normal but varicose by treatment, and degenerated fibres. In consequence, we have not made use of this for purposes of counting the number of nerve fibres.

We may take as a starting point for description the plexus in Exp. 4, in which all the sacral nerves were cut peripherally of the posterior root ganglia.

A sketch of this is given in Fig. 1.

In Exp. 3 on the *left side* (2nd and 3rd sacral nerves cut peripherally of the spinal ganglia) the condition of the plexus was practically the same as that given in Fig. 1. The pelvic nerve contained 16 medullated fibres of 2 to 3 μ diameter, and 1 fibre of 4 to 5 μ diameter. The main branches to the rectum corresponding to the four branches represented

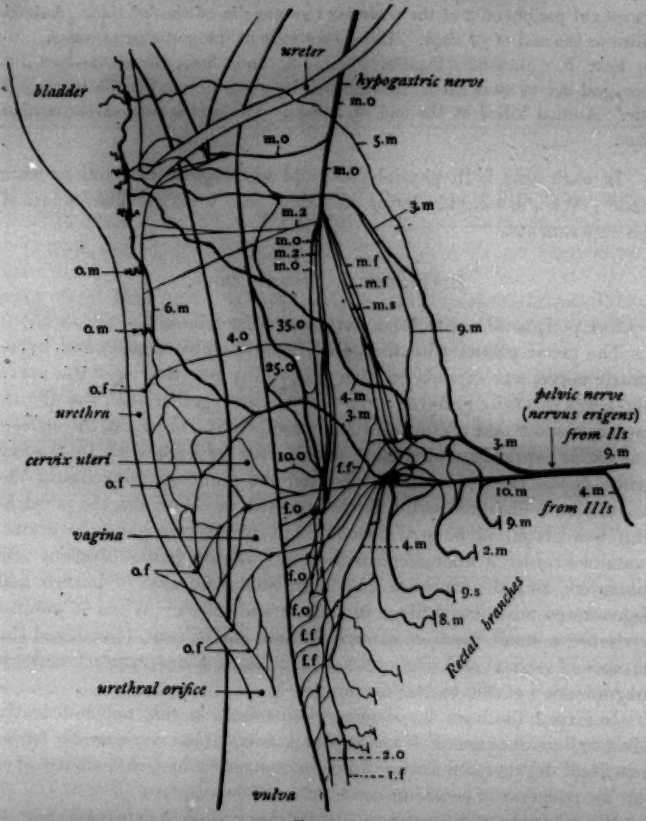

FIG. 1.

Left pelvic plexus of cat (No. 4). The three sacral nerves and the 1st coccygeal nerve were cut peripherally of the spinal ganglia. 11 days allowed for degeneration. The plexus had many small strands, which we have not represented, the medullated fibres in an omitted strand we have counted in the figure as belonging to the strand nearest to it.

The numbers placed opposite each strand show the number of normal and of degenerated fibres present in it; the number of normal fibres being placed first, thus 35.0 indicates that the strand contained 35 normal fibres and not one degenerated. We employ similarly *m* for many, *s* for some, and *f* for few, when we have not thought it worth while to count exactly how many fibres were present.

m signifies more than 25 fibres,
s ,, 10 to 25,
f ,, 9 or less.

Thus 9.*m* indicates that 9 sound, and more than 25 degenerated fibres were present in the strand.

in Fig. 1 near the pelvic nerve, contained 31 small sound medullated fibres, and a considerably larger number of degenerated. In the two more posterior branches, there were 4 sound and about 3 dozen degenerated. In the other filaments running between the bulbus urethrae and the rectum, there were 8 sound and 21 degenerated. Many degenerated fibres ran to the bladder and urethra. No sound fibres were found in the branches entering the urethra, but 5 were found in those entering the prostate. The hypogastric nerve contained 5 degenerated fibres. No degenerated fibres were found close to or in the vas deferens.

In Exp. 5 (rabbit, 1st to 5th sacral nerves cut) the general result was the same, but in comparison very few sound medullated fibres remained. This is connected with the fact that the hypogastric nerve in the rabbit contains comparatively few medullated fibres. The pelvic nerve contained 5 sound fibres.

In Exp. 2 (2nd and 3rd sacral and all coccygeal nerve roots cut), in which the efferent fibres only had degenerated, the pelvic nerve contained about 1100 sound fibres; the main branches to the rectum contained about 100 sound and no degenerated fibres, nor were degenerated fibres found in the more posterior rectal branches. Many degenerated fibres were found in the branches to the bladder; but none were found close to the urethra. The branches entering the uterus contained about 120 sound and no degenerated. No degeneration was found in the hypogastric nerve.

In Exp. 3 on the *right side* (2nd sacral nerve, 3rd sacral roots, cut) there were about 800 sound fibres in the pelvic nerve; 600 degenerated fibres were counted, but this number is certainly much too small, as degenerated fibres are very difficult to count. In the branches to the bladder, urethra and rectum, both sound and degenerated fibres occurred; in the branches to the rectum, the sound fibres were three to five times as numerous as the degenerated. In one of the chief branches to the bladder, but at some distance from it, 45 sound and 170 degenerated fibres were counted. In this branch, most of the fibres were taken on their way to the nerve-cells of the plexus. No degeneration was found in the branches to the vas deferens. The hypogastric nerve contained 2 degenerated fibres.

The innervation of the internal generative organs. As we have indicated in the Figure, no degenerated fibre could be traced to the uterus or to the vagina.

Since the vulva receives both afferent and efferent fibres from the sacral nerves by way of the pelvic plexus, we might expect that at the junction of the vulva and vagina, a few fibres from the vulva might overpass their proper limits and encroach a little on the vagina continuous with it. In Exp. 4 (cf. Fig. 1) there were in fact a few degenerated fibres in the nerve branches running to the junction of the vagina with the vulva; but in Exp. 2 in which efferent fibres only were cut the lower part of the wall of the vagina was carefully examined, and no degenerated fibres found.

There was a similar absence of degenerated fibres in each of the other experiments.

In addition to examining the entering nerve fibres, portions of the vagina and uterus, or of the corresponding organs in the male, were carefully examined, and though sound medullated fibres were found, there was no sign of a degenerated fibre. We regard it then as certain that section of the nerve fibres running from the sacral spinal cord to the pelvic nerve does not cause degeneration of any medullated fibre close to or in the internal generative organs. This, of course, does not inevitably mean that the sacral nerves send neither efferent nor afferent fibres to the internal generative organs. The conclusion only follows if it can be shown that the sacral nerve fibres do not become non-medullated in the stretch intervening between the pelvic nerve and the organs. First as to the efferent fibres. Most of the fibres of the pelvic nerve end in connection with the nerve-cells of the pelvic plexus[1], and there is no necessity for the fibres distally of the nerve-cells to be medullated. If, then, there were efferent sacral nerve fibres to the internal generative organs, and all of them ran to nerve-cells placed at a little distance from these organs, section of the sacral nerve would not lead to any signs of degeneration in the nerve fibres in the organs themselves. But, in the cases of some organs, at any rate, a few fibres continue medullated up to the organs, and become connected with nerve-cells in or on them. This is so with the hypogastric and pelvic nerve fibres to the bladder. Nevertheless it must be allowed that it may be otherwise with the internal generative organs. So that we cannot certainly say more than that the histological facts give no evidence of the existence of sacral efferent fibres to the internal generative organs.

As to the afferent fibres the matter is clearer, since afferent fibres have no peripheral nerve-cells on their course, and can usually be shown to run to the organ they supply without losing their medulla. Thus when all the fibres of the pelvic nerve are degenerated, numerous de-

[1] Cf. Part v. This *Journal*, XIX. 137. 1895.

generated fibres can be followed to the bladder, to the rectum and to the urethra. None can be traced to the uterus or vagina. We may fairly conclude that the sacral nerves send no afferent fibres to these structures. And this is strengthened by the fact that medullated fibres can be traced readily from the hypogastric nerve into the uterus and vagina.

The absence of afferent fibres is again some, though not conclusive, evidence for the absence of efferent fibres.

Constituents of the pelvic nerve (*nervus erigens*). The pelvic nerve in the cat contains normally about 3500 medullated fibres. Of these, one to two dozen are 7 μ to 12 μ in diameter, several hundred are 4 μ to 5 μ in diameter and nearly all the rest are small. In the plexus of Fig. 1, all these fibres were degenerated except 13 small ones. In a similar case given in an earlier Paper[1], we noted that 9 small medullated fibres remained apparently sound in the pelvic nerve. And in the rabbit (Exp. 5) in which the sacral nerves were cut 5 sound fibres remained in the pelvic nerve. These sound fibres could not come from the sacral or 1st coccygeal nerves, since these were cut. The nerves immediately above and below these may be excluded since both physiological and histological evidence shows that they send no fibres to the pelvic nerve. It is unlikely we think that the hypogastric fibres would run so far out of their course. It is possible that a few medullated fibres may run from the sympathetic trunk to the pelvic nerve; but in two cases in which we examined the nerve after previous section of the sympathetic trunk at about the level of the 6th lumbar ganglion, we found no degenerated fibres in it. And it is probable that the ganglia of the pelvic plexus give off some fibres which become medullated. To settle the point the pelvic nerve itself should be cut and subsequently examined. In Exp. 3 the pelvic nerve fibres on the left side had also almost completely degenerated. The nerve contained only 17 sound fibres, one of these being 4 to 5 μ in diameter. We had expected to find a considerable number of sound nerve fibres in this case, for the 1st sacral nerve was uncut and the arrangement of nerves was median. We propose in consequence to make some further experiments upon the relation of the 1st sacral nerve to the nervus erigens.

In Exp. 2 in which the spinal ganglia were intact so that the afferent fibres were not degenerated about 1100 sound fibres were present in the pelvic nerve. Some of these may have been efferent fibres from the 1st sacral nerve, but there could hardly have been many since the sacral

[1] This *Journal*, XVII. 180. 1894.

nerves were of the posterior class. And we may conclude that about one-third of the fibres in the pelvic nerve are afferent fibres.

Innervation of the urethra. A reference to Fig. 1 will show that degeneration of the pelvic nerve was accompanied by degeneration of all the medullated fibres entering the urethra. In other words, few or no medullated fibres run to the urethra from the hypogastric nerves. This is in harmony with the physiological result that stimulation of the hypogastric nerves[1] has little or no effect on the urethra.

Innervation of the rectum. We have mentioned earlier[2] that the first large ganglion—really a group of ganglia—on the course of the pelvic nerve is the chief nerve station for the efferent fibres to the rectum. In Cat No. 2, it appeared to be the exclusive ganglion for the lower part of the rectum, for the degeneration of the efferent fibres caused no degeneration in the main branches to the rectum peripherally of this ganglion. As there were about 100 sound fibres in the three chief rectal branches, these must have been, in the main, sacral sensory fibres. It will be noticed in Fig. 1 that the chief rectal branches contained about 30 sound fibres. Whether these arise from the nerve-cells of the pelvic plexus, or come from the hypogastric nerve, our observations do not decide.

Physiological Observations.

Recovery from anæsthetics. The cats in recovering from the anæsthetics showed more or less signs of hallucination, presumably due to the morphia. This was especially obvious in No. 3. In this animal 2½ cubic centimetres of 2 p.c. morphia were injected subcutaneously. For half-an-hour to an hour after awaking, it was, at first almost incessantly, later at increasing intervals, pouncing on imaginary mice, sometimes missing them, sometimes catching and playing with them. Noises caused by moving about the room, or scratching on the sides of its cage, attracted its attention for a moment only or not at all. It observed with fair accuracy the limits of the cage, rarely dashing against its sides. Sometimes it took for prey some object, such as a piece of flannel, which for a few seconds it would bite and toss from foot to foot.

General effects of section. The animals in the morning after the operation were given milk, and on the following days milk and boiled fish. This they

[1] Part II. This *Journal*, XIX. 73. 1895. Contraction of the muscular sheath of the prostate gland in the dog is caused by stimulating the hypogastric nerve.

[2] Part V. This *Journal*, XIX. 137. 1895.

took readily enough though there was generally some loss of appetite. The healing of the wound and the degeneration of the cut nerves did not, so far as could be seen, cause pain, but the animals were less active when they were allowed to wander about the room, and they slept more than usual.

After section of the 2nd sacral and the lower nerves on both sides, the tail hung flaccid, but very little difference was noticeable in the movements in walking. For the first day or two, the hind feet in walking were lifted rather higher than usual, placed rather mincingly on the ground, and the hind limbs were less fully extended both in standing and walking. These effects decreased in later days.

Section of the 1st sacral in addition to the lower nerves (No. 4) caused more distinct modification in movement, principally in an outward twist of the ankle-joint as the weight of the body came on it.

The large overlapping of the sensory areas of the spinal nerves supplying the skin of the limb, found by Türck and by Sherrington, was shown by the very small area of insensitive skin on the leg, that was caused by section of the 1st and 2nd sacral nerves. The only insensitive region was a small flap of skin on the posterior region of the thigh stretching from the genito-anal region. The insensitive region included the skin bordering the anus and vagina, and stretched a little anteriorly on the root of the tail. Since the section was unilateral, there was opportunity to notice how little the sensory nerves crossed from the uncut to the cut side. In the cases in which the 1st sacral nerve was left intact (Nos. 1, 2 and 3) the skin of the genito-anal region was still sensitive—though less than normal—and touching it caused a reflex contraction of the external anal sphincter. The insensitive region was confined to the tail below the level of the anus.

The effects observed in the rabbit suggest further investigation. It was to be expected that section of the 1st sacral and of the lower nerves should cause greater motor and sensory defects in the rabbit (No. 5) than in the cat (No. 4), since the 1st sacral nerve of the rabbit corresponds, broadly speaking, to the 7th lumbar of the cat. The motor defect was different but not obviously greater in the rabbit. The thigh was turned a little inwards, and the foot and toes were used a little awkwardly.

The sensory defect was greater than in the cat, but it was also greater than we had expected, for we noticed no response on pricking the skin of the foot, or the skin for about 4 centimetres above the ankle, part at least of which region should be innervated by the 7th lumbar nerve. On pinching

between finger and thumb the whole structures of the foot there appeared sometimes to be a slight response. In a cat with the 6th, 7th lumbar and 1st sacral nerves cut[1], there was much greater motor defect, but less sensory defect than in this rabbit.

Section of the 2nd sacral and lower nerves abolished voluntary defæcation and micturition[2]. The arrangement of nerves in these animals was either of the median or posterior class, so that the result is in harmony with our conclusion that in these classes the 1st sacral nerve does not send motor fibres to the intestine or bladder.

Whether excessive accumulation of urine or fæces would have led to spontaneous contraction of the intestine or bladder sufficient to cause defæcation and micturition, was not determined in our experiments since, when the accumulation became marked, we removed the urine and fæces.

In all these cases there was a certain amount of trophic disturbance in the anal region, which was completely absent when the nerves were cut on one side only.

Section of the 1st and lower sacral nerves on one side (No. 4) caused slight constipation and retention of urine, but not to an extent that made it necessary to empty the bladder or the rectum artificially.

In the cats in which the nerves were cut on both sides there was some erection of the hairs of the tail for a day or two after the operation. This was most marked in No. 1. In this animal the hairs were also erect over the lumbar vertebræ. On smoothing them down, they returned quickly for a certain distance, and then more and more slowly. On stroking the animal, the erection of the hairs became greater, but still only occurred on the posterior half of the body. A similar tonic state may occur, after abdominal operations in which for example the rami to the inferior mesenteric ganglia are cut. Occasionally, in these cases, the hairs would become more erect in the lumbar and tail region as the cat approached for the purpose of being stroked. Probably then the degeneration of the afferent fibres caused an increase of excitability in the lumbar pilo-motor centres, and the erection of hairs was of a reflex nature. The point is of some interest since electrical excitation of sensory nerves has not so far been observed to cause a reflex erection of hairs.

In no case did section of the sacral nerves cause any appreciable

[1] Langley. This *Journal*, XVII. 304. 1894.

[2] On this point, however, cp. E. Fuld, *Ueber das Verhalten des Sphincter Ani u. s. w.* Inaug.-Diss. Strassburg, 1895.

modification of the temperature of the feet, though an increase of temperature in the feet was always seen after section of the upper lumbar nerves.

Duration of irritability of the cut nerve fibres. Four days—or more exactly 108 hours—after section of the sacral nerves, most of the fibres of the 2nd and 3rd sacral nerves, whether sacral or visceral, were still irritable. It is known that the duration of irritability of a nerve after its section varies in different animals, and recently Arloing[1] has found very wide variations. We do not know of any observations as to the exact time of disappearance of irritability in cats. In dogs, the irritability is usually said to disappear in four days or less; though occasionally it may continue on into the fifth day[2].

The effects obtained in Cat No. 1 four days after section of the roots of the sacral nerves were:—

			External anal sphincter	External vaginal sphincter	Bladder	Descending colon and rectum
Roots severed 4 days	I Sacral	Extension of limb. Toes separated	Good	Mod. to Good	0	0
	II Sacral	Toes bent and adducted	Moderate*	Slight	Slight	0
	III Sacral	Tail movement	0	0	Mod. to Good	0
	I Coccygeal	Tail movement	0	0	0	0

No certain effect was obtained on the internal anal sphincter.
* The contraction was sometimes confined to the same side.

The effect on the muscles of the limb was not diminished to an appreciable extent; that on the external sphincter of the anus was perhaps a little less than normal; that on the external vaginal sphincter was distinctly diminished. The irritability of the visceral fibres was much less than normal; and no effect could be seen on the descending colon and rectum. It does not however necessarily follow that the nerve fibres to the descending colon and rectum had completely lost their irritability, since these structures were not observed till late in the experiment. (Cp. also below.)

Seven days after section of the nerves (Cat No. 3) the irritability of the cut fibres had completely disappeared. According to Dziedziul[3], the vaso-dilator effect of the nervus erigens lasts for 9 days after section. This statement we cannot confirm. In our experiment, neither the 2nd sacral nor the 3rd had any vaso-dilator or erector action on the penis.

[1] Arloing. *Arch. de Physiol. norm. et path.* 1896, p. 75.
[2] Cf. Howell. This *Journal*, XIII. 335. 1892, and Arloing, *op. cit.*
[3] Dziedziul. *Hermann und Schwalbe's Jahresb.* II. 70. 1880.

The right 1st sacral, which was left intact in the original operation, readily caused erection.

Twelve days after section (Cat No. 2) the state of things was the same as after seven days.

Persistence of irritability in the grey rami accompanying the cut nerves. In the animals in which the cauda equina was cut, stimulation of the nerves near their point of exit from the vertebral canal gave in all cases certain visceral effects, viz. vaso- and viscero-motor effects on the external generative organs (cp. This *Journal*, XVIII. 108, 1895), and erection of the hairs of the tail in the region supplied by the spinal nerve stimulated. (Cp. This *Journal*, XV. 176, 1893.)

Thus in Exp. 3 in which the 2nd sacral and the lower nerves or their roots had been cut for 7 days, the following effects were observed.

II Sacral. Contraction and withdrawal of the penis, contraction of the prepuce. Hairs erect on the base of the tail, a little above the level of the anus.

III Sacral. Slight contraction of penis etc. Erection of hairs on the base of the tail, over and a little below the level of the anus.

I Coccygeal. Erection of hairs for about 8 centimetres below, but slightly overlapping, the area of 3rd sacral nerve.

In the cat in which the nerves had been cut 12 days, the 2nd and 3rd sacral nerves and the 1st coccygeal nerve caused erection of hairs on the tail in the region of distribution of these nerves; the two sacral nerves caused apparently slight pallor of the mucous membrane of the vulva. No certain effect could be observed on the internal sphincter of the anus, but slight effects may have been overlooked since there was strongish spontaneous rhythmic contraction. The effects are due to stimulation of the fibres which run from the sympathetic ganglia to the spinal nerves, and which therefore do not degenerate on section of spinal nerve roots.

Effect of section of the nerves on the action of the uncut nerves. Section of the sacral nerves on one side appears to lower the irritability of the viscera they supply, so that the intact nerves of the opposite side have less effect than usual. Both in cat (No. 4) and in rabbit (No. 5), the sacral nerves of the right side had either a very slight or no effect on the rectum, instead of the normal strong longitudinal contraction. It is true that the rectum was not observed in the earlier nerve-stimulations, but normally the longitudinal contraction can be produced an indefinite number of times, provided there is a sufficient interval between the

successive stimuli. The intact nerves had also somewhat less effect on the bladder, but as the contraction was fairly strong, the difference might possibly be due to individual variation.

Similarly in Cats Nos. 2 and 3, the hypogastric nerves caused less contraction of the bladder than usual. And in our earlier experiments on section of the hypogastric nerves, we noticed that after degeneration of the nerve on one side, the nerve on the opposite had less effect than normal on the internal sphincter of the anus.

Summary of Chief Points.

After section of the sacral nerves peripherally of the posterior root ganglia, many degenerated fibres can be traced in the nerve strands which run to the rectum and bladder, fewer to the urethra and vulva, but none to the internal generative organs (uterus and vagina).

We conclude that the sacral nerves do not send sensory fibres to the internal generative organs.

We have on experimental grounds already urged that the sacral nerves send no efferent fibres to the internal generative organs. The histological observations given here are so far in favour of this conclusion that they show that no efferent sacral fibres reach the internal generative organs as medullated nerve fibres. This however is not in itself decisive, since it is possible that if such fibres existed, they might all be connected with nerve-cells at some little distance from the organs.

Normal medullated fibres can be traced from the hypogastric nerve to the bladder and uterus in considerable numbers, to the vagina in less numbers, but few or none to the urethra. And we have shown earlier that the hypogastric nerve has little or no effect upon the urethra.

The pelvic nerve (nervus erigens) contains in the cat about 3500 medullated fibres. Of these about a third are afferent fibres. It contains about a dozen small medullated fibres which do not degenerate on section of the sacral nerves.

In the branches of the pelvic plexus which run to the rectum, the medullated fibres are almost entirely sacral afferent fibres. The efferent rectal fibres are connected with nerve-cells in the first group of ganglia occurring on the course of the pelvic nerve.

On recovery from ether and morphia, cats may show signs of hallucination, and behave as if they were hunting mice.

Section of the 1st sacral and lower nerves in the cat (nerves

posterior) causes a weakening of the ankle-joint, but very little other change in walking movements; it abolishes sensation in the skin bordering the anus and vagina and in a small piece of skin stretching down the posterior part of the thigh. When the 2nd and lower nerves are cut, the only insensitive region is on the tail.

Section in the cat of the 2nd sacral and lower nerves on one side weakens voluntary defæcation and micturition, section on both sides abolishes it. The operation leads to an increase in the reflex irritability of the pilo-motor centres in the spinal cord. It does not modify appreciably the temperature of the feet.

The irritability of the cut fibres may last 4 days, but disappears in less than 7 days, that of the vaso-dilator fibres of the penis included.

Section of the nerve roots does not abolish the viscero-motor effects of stimulating the nerves near their exit from the spinal canal, *i.e.* the degeneration of the efferent sacral nerves does not lead to degeneration of the sympathetic grey rami which run to the nerves.

Degeneration of the nerves on one side appears to injure the irritability of certain of the structures they supply, so that the nerves on the opposite side have less effect than normal.

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ON THE GRANULAR LEUCOCYTES.

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From the Research Laboratory of the Royal College of Physicians, Edinburgh.

Material and Methods.
The Relations of the different varieties of Leucocytes.
The Acidophile group of Leucocytes (Oxyphile and Eosinophile).
The Basophile Leucocytes.
The significance of the enlarged microsomes or "granules."
The physical relations of nucleus and cell-body in Leucocytes.
Summary.

Material and Methods.

During the past seven years I have accumulated a mass of histological material, consisting of everything which bears upon the study of leucocytes. I have therefore preparations of all the adenoid and lymphatic organs, not only in their adult condition, but also in various stages of development, and taken both from human subjects and from all the animals which are usually employed for histological study. In addition I have made use of a large mass of pathological specimens and of blood preparations.

This material had been prepared in many different ways; I think indeed in most of the ways which are recognised as useful by histologists, and the whole of it was subjected to re-examination for the purposes of this paper. I found however that there were still points on which I could not pronounce definitely from these older specimens, for example, the facts regarding the centrosomes, and I therefore prepared another set of specimens, all intended for examination with the very highest possible powers of the microscope, and treated accordingly. Sections which give a clear picture when examined with powers of from 500 to 1000 are often almost useless when a magnification of 2000 or more is employed.

I have mainly studied, for all finer points in the structure of the eosinophile cells and of leucocytes generally, sections of the following organs and tissues, and coverglass preparations. Sections were taken from the bone-marrow and small intestine of the rabbit, through the liver of rabbit-fœtuses, the ovary of the hen, certain carcinomata in the stroma of which leucocytes were specially abundant, the gills and foot of the salt-water mussel, the tongue, spleen, intestine and heart of the frog, the liver, intestine, heart and other organs of the newt. The coverglass preparations were made from blood of the human being in various normal and pathological states, especially in leucocythæmia, and from the other animals above named, especially the frog and newt. I also made preparations of pus, and did not neglect the examination of leucocytes in the fresh state, in blood and pus.

For the basophile leucocytes I took portions of the mesentery or parietal peritoneum of the newt, frog, guinea-pig, rat, rabbit (adult, young, and fœtus), cat (adult and young), hen, human being (new-born child and fœtuses), in all cases using several animals and several pieces of the membrane. The sections of the organs of the frog and newt also showed large numbers of basophile cells.

These three sets of preparations, sections, coverglasses, and membranes, had of course to be treated to a certain extent in different ways, at least up to the point of staining, and it will be best to take up the method of coverglass preparation first, as that is the one which has been most generally used in the study of the "granular leucocytes."

Coverglass method. This was introduced into histology by Ehrlich[4]. In its earliest form the method consisted merely in allowing a thin layer of blood to dry at the temperature of the room, and then staining it in various ways. Later, in order to preserve the hæmoglobin of the red corpuscles, Ehrlich followed up the drying by exposing the coverglass to the action of dry heat (110°—120° C.) for two hours or more.

The whole of his classification of leucocytes rests upon the assumption that the granules which are stained differently by different dyes are formed of different chemical substances, and the trend of his work has been in the direction of a chemical rather than a morphological differentiation.

Griesbach[5] has entered a strong protest against Ehrlich's method, and he declares that characteristics and differences which are observed in unfixed cells, for instance in cells which have been dried, with or without the use of reagents or stains, cannot be regarded as

pre-existing till it is ascertained that they can be made visible in the same way in cells which have been instantaneously killed.

So long as we merely wished to know whether granules in a cell would stain with eosin or with dahlia it was possible to treat the cell as Ehrlich does, but if we wish in addition to understand the relation of the granules to other and more delicate parts of the cell, we must use finer methods. Experience has shown that whether drying only affects the cell by removing water from it or not (and it must be remembered that we have no absolute proof that this is the only change which takes place), this method certainly does not preserve the minute structure of the cell, either as regards the structure of the nucleus or the minute arrangements in the cell-body itself, in a satisfactory way. The modification of Ehrlich's method, which consists in fixing with a mixture of equal parts of alcohol and ether after drying, I have found to act no better than the original method.

The plan I now use is that advocated by Muir[13]. As soon as the drop of blood, which is to be drawn in the usual way, appears on the finger-tip, the coverglass is applied, and a thin layer of blood is attached to this by moving it rapidly over the extreme apex of the drop. The coverglass is at once placed on the surface of a large quantity of normal salt-solution saturated with corrosive sublimate by boiling. I do not usually spread out the layer of blood by pressure in any way, as I find that the corpuscles tend to be deformed by this procedure. If one makes a sufficient number of preparations it matters little if the layer of blood be too thick at one or two points. In cases indeed where the leucocytes are not numerous, as in the blood of some frogs, it is a positive advantage to have the cells lying close together. When the blood has been treated with the sublimate solution for at least half-an-hour (24 hours or even a longer sojourn does not injure the preparation), the sublimate must be removed by washing first with 0·75 % salt-solution, and then with very slowly increasing strengths of alcohol, to which salt is added so that the normal proportion is preserved. We may, when the higher strengths of spirit are reached, use the addition of a little tincture of iodine to the alcohol to remove the sublimate. If it is desired to preserve the hæmoglobin great care must be taken to add very little iodine, as with anything more than a trace, the weaker of the red corpuscles are apt to be laked. The leucocytes are however in no danger even if strong solutions of iodine are used. When 95 % alcohol has once been reached the coverglasses may be treated with any stain exactly as if they were sections fixed to

a slide, and may of course be taken back through water for this purpose. The adhesion of the corpuscular elements to the glass is perfect, and what is more important the fixation of the cells is quite satisfactory, and minute structures are completely preserved.

I have also tried the other coverglass method which is sometimes used, of fixing the film at once in absolute alcohol. As far as the leucocytes are concerned the results are very fair, though not so good as with sublimate. In order to preserve the red corpuscles one must however use stains of high alcoholic strength, which is not always desirable.

Ehrlich and his followers declare that sublimate increases the acidophile affinity of the granules. It certainly does so, but I believe only because it fixes them better than drying.

Sections. The organs I have named above were almost all fixed with sublimate. The special reason that induced me to employ it was the fact that when it has once been thoroughly removed from the tissues, they can be stained in any way that is desired. The only other really good fixative that allows the same untrammelled choice of stains is absolute alcohol, and I have used this as a control in some cases. There is certainly however a greater tendency to shrinking in the alcohol preparations than in those made with sublimate. I have also used, but only for purposes of control, the chromic and osmic or platinic mixtures. No amount of washing will remove these substances completely from the tissues, chemical compounds are formed by them which may not represent the combinations in the living cells, and the subsequent freedom of choice in the matter of stains is seriously interfered with.

After fixation, the sublimate was removed from the pieces of tissue in the usual way, by washing in water, and by adding tincture of iodine to the alcohols used for hardening. From absolute alcohol the tissues were taken through cedar oil into paraffin, and were cut with the rocking microtome. The sections were fixed to the slide by my water method.

M. Heidenhain[21] insists that in studying the centrosomes of the leucocytes in the rabbit's bone-marrow, the maximum thickness of section must be 4μ, while his usual working thickness was 3μ. I found, in following out his observations by his own methods, that this was in fact the thickness best suited to the purpose; the sections were thin enough to allow accurate definition, and thick enough to permit accurate orientation and to show a sufficient number of centrosomes in each field.

But when dealing with the eosinophile cells, especially by Heidenhain's method, I found that with sections of this thickness the granules often formed a mass impenetrable to the lens. I therefore cut a number of sections of bone-marrow with one tooth of the rocking microtome. This gives a section of the theoretical thickness of 0·625μ, but perhaps if we say that the sections are 1μ in thickness it will more nearly correspond to the actual fact. These sections when treated by the iron-hæmatoxylin method gave me an insight into the relations of the granules which I was then able to apply to the interpretation of thicker sections. It is however only exceptionally possible to get good sections of this degree of fineness with the other tissues studied. Most of these were cut either with 3 or with 5 teeth, giving a calculated thickness respectively of 1·875μ and of 3·125μ.

Membranes. Most of these were fixed with sublimate, some however with absolute alcohol. The former gave the better preparations. Some of the membranes were stretched on cardboard rings before being put into the fixing agent, others were fixed *in situ* in the animal, while others were removed from the body, and put into the fluid without any special attempt to stretch them out. I do not think it matters much which method is used, as there is so much elastic tissue in the peritoneal wall that a certain amount of folding is unavoidable, do what one will in the way of stretching out. The folding is always microscopic rather than macroscopic, if the preparation has been properly made, and is of very little consequence, as the ramifications of the large basophile cells never, by any chance, lie absolutely in one plane, and one must consequently in any case be constantly altering the focus when examining them with high powers.

The pieces of membrane were treated as regards hardening exactly as if they had been blocks of tissue, and as regards staining and mounting as if they had been sections cut with the freezing microtome.

In order to see more of the nuclei of the basophile cells than can always be made out in flat preparations, I cut sections of these membranes as a control. Relatively large pieces of mesentery were taken and allowed to fall together till they looked like cylindrical pieces of tissue. These were then fixed, taken through paraffin, and cut across the cylinder.

Stains. I have experimented with almost all the stains in the modern histological armamentarium, but I found that a very few were sufficient for my purpose. Those which I have principally used were the Ehrlich-Biondi solution, Heidenhain's iron-hæmatoxylin,

Mayer's acid alum hæmatein or Ehrlich's hæmatoxylin followed by eosin, acid fuchsin, or some other acid stain or combination of stains, and methylene-blue alone or with a contrast stain.

Besides the Ehrlich-Biondi mixture I have tried a number of similar combinations, Ehrlich's most recent "triacid" stain (Reinbach[27] Aronson and Philip's so-called neutral stain[28], and others (cf. Bergonzini[29], Prenant[30]), but I have found none of these so good as the Ehrlich-Biondi. That mixture can be used in so many different ways by making slight modifications in the process that it can be adapted to every object. It may be acidified before use, as Heidenhain[31] does, or the stain may be differentiated by a slightly acid medium such as methylated spirit instead of being washed merely with distilled water. The quality of the stain may be modified by the previous use of tincture of iodine, by using the dye diluted to $\frac{1}{10}$ or $\frac{1}{100}$ of its original strength and allowing it to act for 24 hours, or by using it undiluted and staining for a shorter time. In the last case from 10 to 30 minutes is the length of time usually necessary to get a stain deep enough for use with high powers. As Heidenhain points out[32] the mixture contains not only one basic stain, methyl green, and two acid stains, acid fuchsin and orange, but also probably two neutral stains, resulting from the mixture of the acid stains and the basic one. These neutral dyes one cannot recognise in the finished preparations when dilute solutions are allowed to act for a long time, but they are often to be seen when the undiluted dye has been allowed to give a deep stain, and when the after-treatment has been a very short washing with distilled water, rapid dehydration with absolute alcohol, clearing with xylol and mounting in xylol balsam. This is the method which gives the greatest amount of differentiation, and allows the fullest advantage to be taken of the selective powers of the stain. If it be used very dilute, or if any considerable amount of acidification be employed either before or after staining, the result is very much the same as if methyl-green and acid fuchsin had been used one after the other. Of course this is excellent for many purposes, but the finer shades of differentiation are lost, and the experiments of Zacharias[33], Lilienfeld[34] and others have shown that these minute differences in colour probably or possibly depend on difference in chemical constitution. They cannot therefore be neglected.

M. Heidenhain observed incidentally that by his iron-hæmatoxylin method the granules of the eosinophile leucocytes were stained a deep black, and I have systematically made use of this reaction. I find that

the previous staining with Bordeaux R., anilin blue, or acid fuchsin, which is so valuable an aid in examining the centrosomes, is rather a hindrance in working with the granules, and I have generally preferred the process as he originally published it[39]. The time of staining in the ammonio-ferric alum and in the ½% hæmatoxylin solution may be varied at pleasure; half-an-hour is however the minimum time in each of the solutions, and generally speaking the longer the better. It seems to be of little importance whether one uses 1½% or a 3% solution of the ferric alum as the mordant, but one should never, I think, use a stronger solution than 1½% for decolorization, as the precision of the stain depends greatly on the slowness of washing out. The point at which this process should be stopped is earlier than that required for the centrosomes; the moment when the nuclear structure is clear and distinct, but before any part of it has begun to be decolorized, is that which is most satisfactory for the granules.

I have worked through the processes of staining with eosin in saturated watery, glycerine, and alcoholic solutions which Kanthack and Hardy[38], following Ehrlich, have recently employed. It is quite true that by using eosin in this way three sets of granules may be made out. The granules which are most strongly eosinophile are those which stain with all three solutions, while the granules which are only slightly eosinophile stain only with the watery solution. But the distinction is a purely arbitrary one, for there are many shades of difference in each class which would if put together make a scale uniting the ends of the series without any break. Moreover exactly the same result may be gained by using the Ehrlich-Biondi mixture, for example, where the different classes of cells, or rather of granules, distinguish themselves by the amount and kind of the acid stain which they take up, and where, instead of requiring to compare different preparations, one can see the cells side by side.

In examining the basophile cells I used almost entirely various methylene-blue solutions. The original methods of Ehrlich and his school involved the addition of a large amount of acetic acid, sometimes as much as 12½%, to a solution of a basic dye such as dahlia, or else the extraction of the basic stain by a strong acid. The basophile granules retain the stain under this treatment, it is true, and one can then very easily pick them out in the preparation, but not only is the rest of the preparation rendered colourless or nearly so, but the nuclei of the basophile cells are themselves decolorized. As it was my aim to see as much as possible in the cells, I soon ceased to use these "acid"

methods, as one may call them. It was known to Westphal[14] that the basophile granules stained metachromatically with solutions of methyl-green. This was due probably to the presence of methyl-violet as an impurity, so that the nuclei were stained green, the granules violet. Unna has since shown[15] that methylene-blue solutions, if they are kept a long time and especially if they have been made slightly alkaline to begin with, "ripen" in course of time. The ripening consists in the taking up by the alkali of carbonic acid from the air, and the consequent slow formation of methylene-red at the expense of the methylene-blue. Most basophile leucocytes, when stained with such a solution, present blue nuclei and red granules, and as the rest of the preparation is stained in various shades of blue, the basophile cells are easily picked out.

Unna has shown that solutions of methylene-blue may be ripened artificially, and I have found such solutions give very good results. I possessed a large quantity of carbol-methylene-blue solution made about two years earlier according to Kühne's formula. It gave a most excellent metachromatic differentiation; I used it as a standard solution by which to compare different preparations. The mode of procedure was simple. The preparations were brought from alcohol into tap-water, and thence into the methylene-blue solution. There they remained a variable time—from 2 minutes to 12 hours,—they were then washed out in tap-water till no more colour came away, and then were always differentiated in methylated spirit. This part of the process must be followed under the microscope. When the desired point is reached—the moment when nothing is visibly stained in the preparation but the nuclei, but when the stain in them is still deep,—the preparation is rapidly dehydrated in absolute alcohol, cleared in cedar oil and xylol and mounted in balsam. I preferred spirit to any other differentiator because of its slowness of action and because it partly dehydrates at the same time, so that only a small amount of absolute alcohol is required to complete the process. The risk of weakening the stain is thus minimised.

In using methylene-blue with sublimate preparations it is important to see, first, that the sublimate is entirely removed from the tissues, and, second, that the iodine used to remove the sublimate is also thoroughly washed out, as the presence of either of them causes precipitation.

The basophile cells of the dog's intestinal villi, when fixed with absolute alcohol and stained with alcoholic methylene-blue[16], give

exactly the same results as to mitoma and granules as other basophiles.

Examination and drawing of the preparations. To the Royal Society I owed the use of a Zeiss's apochromatic homog. immers. lens of 2·0 mm. equiv. focus, and aperture of 1·40, and of the necessary compensating oculars. I found this lens much better than any other I have had an opportunity of examining.

I have used gas entirely as a light-source, and found that an Argand burner corrected by a light-blue glass disc under the condenser was much to be preferred to daylight. Of late I have adopted Heidenhain's suggestion, and have used a layer of cedar oil between the condenser and the lower surface of the slide, which gives a much better illumination and definition. The amount of light used for working with the plasmic structures was always the smallest possible. A flood of light drowns them completely.

In drawing I have generally put in the outline and placed the larger granules with the help of a Zeiss's camera lucida, but with a magnification of 2250 it is not possible to fill in all the finer details in that way, and they have been inserted with the free hand.

The Relations of the different varieties of Leucocytes.

These have been discussed by me in earlier papers [8 and 20], but as new facts and new views have since then been published, it may be well to restate my present opinion.

Throughout this paper I propose to use the terms "lymphocyte," "hyaline," and "basophile" in the sense in which they are usually applied to leucocytes. Kanthack and Hardy [22] and Sherrington [23] have pointed out that the so-called "neutrophile" and "amphophile" granules are really "oxyphile," and I shall speak of them by the latter term. The cells bearing these granules are, I believe, the precursors of the eosinophile cells, and I propose to group these two sets of cells in one series under the name of "acidophile" cells. The term is an unfortunate one, but I know of no better one. The name "eosinophile" is too firmly attached to the coarsely granular cells to be easily displaced, and it is better to retain it and to use "oxyphile" for the finely granular cells, than to employ the cumbrous phrases "finely granular oxyphile" and "coarsely granular oxyphile." I attach comparatively little importance to the names that are used; it will be seen that I regard the different forms as different stages in leucocyte development, and not as cells different in kind.

It is not necessary in the present connection to prove that leucocytes are cells specifically distinct on the one hand from the cells of connective tissue, on the other from nucleated red blood-corpuscles.

It is now universally agreed that leucocytes multiply by mitosis. If amitosis occurs at all it is extremely rare, and probably pathological. A leucocyte of any variety may divide, provided the cell has attained a sufficient size, but there are certain forms which do so only rarely. One may say generally that the more specialized in any direction the leucocyte becomes, the less likely is it to divide. Thus mitosis has only once been seen in one of the large basophile cells; it has never been seen, to my knowledge, in the large macrophages of the adenoid organs, which are the extremes of the hyaline or stationary series, and it occurs with comparative infrequency in the eosinophile cells. The cells which one sees dividing or about to divide have generally the appearance of medium-sized hyaline cells, with a relatively large rounded nucleus and a comparatively small cell-body, in which the mitoma is not easily made out. But there is no doubt that cells with horseshoe-shaped nuclei divide, and that the nuclei may even advance as far as the spirem stage without altering their shape. Cells with more markedly polymorphous nuclei, as for instance the ordinary oxyphile cells, certainly divide also, but they seem generally to go through a preliminary resting stage, in which the polymorphous nucleus returns to the rounded form. We know that this may happen in such creatures as Vorticella; the polymorphous nucleus has been observed to return to the rounded form in leucocytes by several observers (Arnold, Carnoy, Reinke, Sherrington); and I have given reasons for believing that this must frequently happen in the "germ-centres" in the adenoid organs[10], in which the mitosis of leucocytes most frequently occurs. One often sees mitotic figures in leucocytes in which the cell-body at the margin shows a fairly evident mitoma, like that of the oxyphile cells.

During the process of mitosis the original shape of the nucleus is of course altogether lost, and on reconstitution the daughter-nuclei assume the spherical shape in all cases, and we have the young leucocyte, which is known as a "lymphocyte." Though the amount of cell-substance which surrounds the nucleus of these cells varies in different cases, probably in accordance with the size of the cell from which they sprung, it is always too small to allow one to make out any structure in it except the presence of the microcentre with its (usually) two centrosomes, which can be seen in favourably placed cells. The only exception to this is the rare one in which one finds a young cell which

has resulted from the division of an eosinophile cell. Here the nucleus is exactly like that of an ordinary lymphocyte, but in the cell-body are a few eosinophile granules, though these are often surprisingly few when one remembers the number in the mother-cells.

One can trace the further growth of the lymphocyte best in the adenoid organs, and nowhere better than in the tonsil. The lymphocytes are here found crowded closely together at the margins of the germ-centres, retained there by the special arrangement of connective tissue. From this position they pass out gradually either into a lymphatic vessel or into the general adenoid mass of the organ. In the latter situation the leucocytes are mainly of the hyaline variety, relatively stationary cells, and one can trace every gradation from the lymphocytes to the large macrophages which lie under the epithelium. If one looks on the other hand at the cells passing through the epithelium into the crypts, it will be found that the cells passing from the side of a germ-centre—or follicle—nearest the crypt, where the epithelium has been almost entirely removed by the action of the leucocytes, and where consequently there is no difficulty in the passage, are almost all either lymphocytes or hyaline cells. These are possibly carried out by the *vis a tergo* of exudation. Where however the epithelium is largely intact, so that its passage involves individual movement on the part of the leucocytes, it will be found that the leucocytes underlying the epithelium are hyaline, that as they pass through the epithelium the cells and nuclei lengthen, and assume whatever shape is necessary, and when they reach the surface the nuclei are polymorphous, and the cells absolutely indistinguishable from the oxyphile leucocytes of the blood. That is to say, the leucocytes have adopted the form which is most suitable to their environment. Those which have remained stationary in the adenoid tissue, where they are richly supplied with nutriment, and where there is no need to seek for it, have retained the hyaline character with which they started; those which have left the adenoid organ, and have encountered difficulty in doing so, have undergone changes in nucleus and cell-body which, I believe, are closely connected with amœboid movement.

The lymphocytes which pass from the germ-centres of the tonsils and other adenoid organs into the lymph-vessels, are of course ultimately carried with the lymph into the blood. Many of them are arrested temporarily or permanently in the lymph-glands on the way, may develope there into one or other of the more highly specialized types of cells, and only thereafter be passed on into the blood. The cells produced by

mitosis in the lymph-glands of course add to the number of immigrants into the blood-vessels.

Some observers still divide leucocytes into definite classes, and maintain that these have no relations of a genetic kind with one another. Such classifications are purely artificial, for the following reasons, in addition to the facts I have already cited:—

(1) All leucocytes when they divide give rise to cells having the character of lymphocytes. The exceptional facts as to eosinophile and basophile cells only give greater relief to this.

(2) All the lymphatic organs furnish lymphocytes to the lymph and blood.

(3) The shape of the nucleus which the classifiers have regarded as constant, and have used as a characteristic of primary importance, has been shown to be extremely variable, and to be in a state of continual change. Thus no one shape of nucleus is associated invariably with any one type of cell-body, or with granules of a special kind.

Nuclei may be rounded or ovoid in the following cases:—

I. Lymphocytes.
II. Cells about to divide.
III. Cells at rest when the diameter of the nucleus is less than half the diameter of the whole cell. (M. Heidenhain.)
IV. Cells in movement when the proportion in size is as in III., when the movement is slight, and does not involve the passage of obstacles.

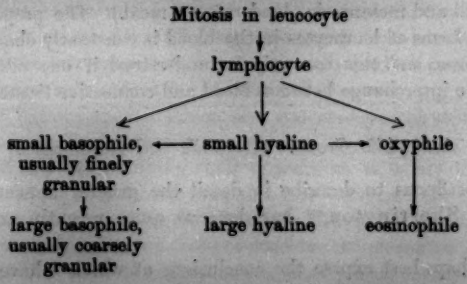
On the other hand, nuclei may be polymorphous in the following cases:

I. Cells at rest, when the diameter of the nucleus has been greater than half the diameter of the whole cell; here the tendency is to deformation in the shape of a horseshoe, *e.g.* many cells in bone-marrow.
II. Cells in motion, when the shape is governed by the direction of the movement, the nature of the obstacles, and the traction of the cytomitoma, *e.g.* cells wandering through epithelium or connective tissue.
III. Cells at rest, where the nucleus has been previously deformed as in II., and where the centrosomes have reached the centre of the cells, *e.g.* many oxyphiles and eosinophiles.
IV. Cells at rest, which are undergoing changes connected with metabolism (?), *e.g.* giant-cells of bone-marrow.

Thus the shape of the nucleus gives us valuable information as to the history and present state of each individual cell, but can hardly be used as a distinguishing feature. To say that a nucleus is "characteristically trilobed," as Kanthack and Hardy[29] do, or to maintain, as Hardy has done in a later paper[30], that one can tell by the shape of the nucleus alone from what animal a cell is derived, is quite unwarrantable.

(4) The following pages and drawings will show that the granules in the cell-body, whose presence and character, or absence, have also been made a means of classification, are plasmatic structures, not paraplastic, as has been hitherto believed. They are an index of specialization in the cells, but this specialization is the result of gradual development in any given case, and there are countless transition-forms between the ordinary types. I do not mean that the fully developed cells at the extremities of the series are interchangeable. So long as the cell is not too completely specialized an alteration in the environment may cause an alteration in its character, but after a certain point is reached, a point as yet unknown, this probably becomes impossible.

The following scheme will illustrate my meaning:—



Of course I do not consider that every oxyphile cell, for example, becomes an eosinophile cell, probably the transition from one to the other goes on mainly in certain foci, of which the best known is the bone-marrow. Great numbers of oxyphiles are lost, probably in the tissues, and certainly in the lumen of the alimentary and respiratory tracts, and on the mucous surfaces generally.

It will almost be evident from what I have already said, how futile it is to attempt to divide leucocytes into fixed and definite varieties, and still more so into hæmal and cœlomic groups, as Kanthack and

Hardy[35] have recently tried to do. The following further reasons may be adduced against their contentions:

(1) The classification depends almost entirely on coverglass specimens of blood and of peritoneal fluid; the adenoid organs, the true home of leucocytes, do not seem to have been examined. Leucocytes wander into and out of the blood-vessels just as they wander through any other connective-tissue spaces. The physical conditions of the blood-vessels determine to a certain extent the size of leucocyte to be found in the blood; very large cells cannot pass the capillaries.

(2) Leucocytes are not primarily blood-cells at all; embryos are several days old before these cells appear in the blood.

(3) Their classification fails, since they do not show by what means the numbers of their varieties of cells are kept up. They describe immature forms of some of their varieties, but give no hint of their place or mode of origin. They consider that the lymphocytes give rise only to the hyaline cells, but do not explain the curious fact, that hyaline cells form only 2% of the leucocytes in the blood, while lymphocytes are always present in the proportion of at least 20 to 30%, and may even reach 70 to 80% after a meal. (This is of course due to the enormous increase in the amount of mitotic reproduction in the intestinal wall and mesenteric glands after a meal.) The proportion of the different forms of leucocytes in the blood is constantly changing, as everyone allows, and this can only be understood if one admits the freest possible interchange between blood and connective tissue.

The Acidophile Group. (Oxyphile and Eosinophile.)

It is superfluous to describe in detail the general appearance of these cells. Sherrington[36] has done so quite recently and quite accurately.

I can perhaps best expose the conclusions at which I have arrived regarding the derivation of the large eosinophiles if I trace the development of the cell from the lymphocyte. This cell consists, as is well known, of a spherical nucleus with a very thin shell of protoplasm surrounding it; its two centrosomes (usually) lie at the side of the nucleus in a little excrescence of the cell-body. The cell grows by the rapid increase in size of its cell-body and by a more gradual increase in size of the nucleus. The growth in size of the nucleus is due less to an increase in the amount of chromatin than to an increased amount of the inter-reticular substance, so that the chromatin strands are

separated more widely. Some observers have given the name "hyaline" leucocyte to this stage.

It must not however be thought that the hyaline cell-body is structureless. The constant presence of the microcentre alone would prove the contrary, and though no hint of structure can be seen in the lymphocytes, or even in the smaller hyaline cells, yet as they become larger, one can see the ordinary network of protoplasm (see Figs. 7 and 9). In the hyaline cells the nucleus may remain round or may alter its shape according to Heidenhain's law, that is, according to the relation of its diameter to the diameter of the whole cell (Figs. 1, 7, 9).

The process of change from the small hyaline cell to the finely granular oxyphile cell is associated with an increase in the visibility of the mitoma. It is this, in the early stages, rather than the actual appearance of granules that gives the finely granular appearance to the cell-body. Along with this goes the deformation of the nucleus by amœboid movements, and the ordinary polymorphous form is soon reached. When these forms are seen in the blood the cells are generally approximately spherical, and the microcentre is situated in the middle of the cell (Figs. 18 and 19).

The centrosomes are small, too small for one to be sure of their number as a rule, and I have found them stain better with the unmodified Ehrlich-Biondi stain than with iron-hæmatoxylin. The limits of the astrosphere are not usually well-marked, except sometimes in the larger cells (Fig. 19). As the cells become larger the so-called granules become more evident, and one sees clearly that they are, as Heidenhain first surmised, simply the microsomes of the mitoma. The best way of describing their appearance is to say that they look like the knots in a fishing-net; this also expresses the fact that each microsome, in these rounded forms, is the point of attachment for several threads (when we come to consider the elongated basophiles we shall find that there the microsome is often placed simply in the course of a single strand).

Minute as these microsomes are it can already be made out that they vary in size to a certain extent; on the whole, the largest microsomes are towards the periphery of the cell. In the larger cells of this stage the interstitial substance between the threads of the mitoma becomes increased in amount and the strands are consequently placed further apart. The difference is seen in the contrast between Figs. 18 and 19 and in Figs. 2 and 8.

The transition-forms between the finely granular and the coarsely

granular acidophile cells are seen much more frequently in the bone-marrow than in the blood, and it seems certain that both from this source and from mitotic division the main source of the eosinophile cells is in the bone-marrow. They must arise elsewhere however in abundance, for both Schaffer[7] and I[30] have shown that they are present in the thymus and in lymphatic glands before either bone or bone-marrow is properly formed at all, and Engel[19] has seen them in the chick's blood on the 5th day of incubation. In the transition-forms (see Figs. 2, 8, 11) there is little in the general shape of the cell and nucleus to distinguish them from the preceding stage. The real difference is the fact that the mitoma has become much more visible and still more widely spaced, and that some of the microsomes are much larger than others. These larger microsomes take up eosin and iron-hæmatoxylin much more deeply than the smaller granules, and it is at this stage too that the threads of the mitoma can first be said to stain satisfactorily with iron-hæmatoxylin (see Fig. 11).

One can quite well make out all gradations between the large finely granular oxyphile cell with a few large microsomes and the fully developed eosinophile cell.

When one examines an entire eosinophile cell all that can be seen is a mass of granules in which the optical section is so confused by the granules above and below that it is impossible to make out any mitoma with certainty: when I began this research I was familiar with such appearances as this only and I was fully prepared to find that the granules were paraplastic. I do not indeed think it possible to make out from specimens stained with acid dyes alone the relation of the granules to the mitoma. The reason for this is that the threads of the cell network do not stain very deeply with the acid dye, and the bright refraction of the granules blinds the eye to the presence of the threads. It is for this reason that Heidenhain's iron-hæmatoxylin used with thin sections is so valuable a process. The granules are stained opaquely in shades of black and grey so that their refraction is to a great extent destroyed, and the mitoma itself takes up the iron. This it does to an extent varying not only in different cells, but in different parts of the same cell. With this method one finds then that the eosinophile granules are not isolated bodies lying in a structureless matrix, but that they are greatly enlarged microsomes of an orderly mitoma. I have found this to hold good of all the eosinophile cells which I have seen, both in the blood and elsewhere, and in all the animals I have examined (Figs. 10, 12, 13, 14, 15, 16). The size of the

granules is very far from being the same even within the limits of the same cell, although some authors have maintained that in each cell the granules are of the same size. The mistake has arisen from looking only at the entire cells, and the peripheral granules are often very nearly the same in size. A glance however at my Figs. 10, 12, 16, will show how great the range of difference is. Heidenhain believes that the granules are arranged radially to the astrosphere, with the smallest granules next the sphere, the largest at the periphery; and he has pointed out, which I can fully confirm, that there are never any granules within the astrosphere itself. The microcentre does not differ from that of other leucocytes, but the limits of the astrosphere are always very evident because of the granules which surround it (Figs. 10, 12, 16). The radii within the sphere are usually pretty well stained by the iron-hæmatoxylin. I have tried to make out Heidenhain's radial arrangement of the granules but have not satisfied myself that it really exists. Appearances are often seen (as in Fig. 16) which suggest it, but I believe that here as in the other leucocytes of which I have spoken the mitoma forms a true network or sponge. There certainly are constantly connecting threads running between the microsomes; I have taken the greatest pains in the drawings only to insert those threads of which I could be certain.

As a general rule the smaller microsomes are those nearest the astrosphere, but the rule is by no means absolute. Heidenhain argues from the fact that the largest granules are at the periphery that they are older in time of formation than those near the microcentre, but it is evident from the context that he thought of them at the time as paraplastic.

The interstitial substance between the threads of the mitoma is structureless, but it is also slightly acidophile. This is in marked contrast to the interstitial substances in the basophiles, which has no special tendency to take up stains.

The mitoma in the eosinophile cell is especially well seen in those of the newt's blood. These cells are markedly amœboid and have the habit of throwing out circular pseudopodia which are often connected to the main part of the cell only by a very delicate thread. Two such cells are shown in Figs. 3 and 6, and it is evident that the threads are often broken through and the spherical portion of the cell-body set free, as the blood contains a large number of them. The mitoma in these is often very clear, as may be seen from Fig. 5 and from Fig. 17, where one of these bodies has been ingested by a "hyaline" leucocyte.

When the eosinophile cells are found degenerated in blood or pus examined in the fresh state, the granules are always in Brownian movement. The cell-membrane, or outer layer of the cell-substance, must here be very resistant though it is optically structureless, as the cells certainly do not break up until very late in the process of degeneration. This can be seen better in fixed and stained specimens, where one may sometimes find eosinophile cells in which the structure can be barely recognised, and where yet the shape of the original cell is perfectly retained.

The nucleus dies by chromatolysis, as the nuclei of other leucocytes do; there is nothing special in the process here. In the cell-body the granules, which are usually large in the over-ripe cells, gradually lose their affinity for acid stains and for iron-hæmatoxylin; they stain with the former (the red stains) a pale pink, with the latter, of different shades of grey. The threads of the mitoma running between the granules soon cease to be evident and the sharp contour of the granules themselves is lost; their edges blur, and the cell-body is at last a mere mass of irregularly shaped pieces of pink or grey protoplasm.

The process can be followed with special ease in the detached pseudopodia of the newt's eosinophile cells, which, as I have remarked, are to be found scattered through the blood. As there is neither nucleus nor centrosome in these portions they soon degenerate, but like eosinophile cells elsewhere they retain their integrity to the last. Fig. 5 shows one of them apparently soon after its detachment from the leucocyte, while the mitoma is still very evident. Indeed, it has seemed to me as although in the earlier stages of degeneration the mitoma were even more evident than in the healthy cell. The reason of this may perhaps be that by imbibition of fluid the amount of interstitial substance is increased, and the threads of the mitoma are thus separated and seen more clearly. In support of this I may note that I have often seen the mitoma similarly evident in leucocytes in urine when the fluid was examined immediately after being passed. M. Heidenhain has noted that the microcentre and astrosphere persist long in degenerating eosinophile cells.

When the finely granular oxyphile cells degenerate the process seems to be exactly the same as in the eosinophile cells, but the microsomes are so minute that it is excessively difficult to follow out the change in detail.

The Basophile Leucocytes.

These, it is known, vary in size from cells little larger than the ordinary lymphocyte to some of the very largest to be found in the body. The smallest cells are most often to be found in the blood, both normally and in greater number in some forms of leucocythæmia. They are also found however in connective tissue, and I have been able most easily to find them in the mesentery of the rabbit. The larger the cells the rarer they are in the blood. I take this to be due simply to the fact that cells whose diameter is usually 20 μ, and may far exceed that, would not go farther in the blood-stream than the first capillary.

I consider, contrary to Kanthack and Hardy, that the basophile cells are amœboid. They examined only the basophile cells of the peritoneal fluid. I find, however, that most of these cells are degenerated. The evidence for their amœboid power is the irregularity of their shape in sections of the tissues (cp. Figs. 26, 27, 29, 30, 41), the phenomenon of "Körnchenzerstreuung," and the way in which the cells are scattered throughout the body. It seems to me that such shapes can only be explained by amœboid action. In drawing them they must of course be represented in one plane, but one does not in fact find them arranged in that way. The process of Fig. 30 was turned up into quite another direction. Fig. 29 is the most interesting; the end marked *a* is evidently anterior, and the leucocyte was seen to have been fixed in the act of passing through a narrow hole between two bundles of connective tissue. The nucleus shows in its anterior half that diffuse staining which Arnold long ago pointed out as accompanying amœboid movement, and which probably depends on the squeezing together of the chromatin-containing filaments.

Westphal[46] distinguished three main forms of the large basophile cells, flat, spherical, and spindle-shaped, with two or more processes; he remarks however that this is purely a classification for convenience, as transition-forms of every kind are to be seen also. This I can fully confirm, as I can also his dicta that "in individuals of the same species and same age the distribution, number and size of the cells are similar in similar organs," and that in individuals of different species there are great differences in the number &c., of the cells in the same organs. I may add that certain species show a tendency to have cells of one or other shape or size. The mesentery of the rabbit, for instance, contains, *speaking generally*, small cells, mostly spherical (Figs. 4, 24, 25); of the

guinea-pig, larger spindle-shaped cells (Figs. 29 and 30); of the rat, enormous round and flat cells (Figs. 31, 32); while that of the newt has huge spindle cells which send off often several secondary branches from the primary ones (Fig. 41).

The nucleus shows no such vagaries in form as the cell-body. It is almost always more or less oval; the only serious departures from that form are in leucocytes which from their shape I conclude to be actively moving. This regularity of shape is to be brought into line with the two facts that the basophile cells are not, as a rule, so actively amœboid as the eosinophile cells, and that the amount of cell-body in the basophile cells is, except in the very young cells, very much greater than in the eosinophile cells, while the nuclei do not show such a marked disproportion. In this way the nucleus is always less in diameter than half the diameter of the cell-body and, by Heidenhain's theory, escapes deformation. I shall return to this point when discussing the centrosomes of the basophiles.

It is often totally impossible to see the nucleus of the large basophile cells in the mesentery when the specimen is stained with methylene-blue, the stained granules are crowded so closely round the nucleus as to hide it altogether. (Figs. 31 and 32.) One may however in these cases overcome the difficulty by using the Ehrlich-Biondi stain, or by cutting sections of the folded mesentery. I regard the history of the nucleus as being somewhat as follows. In the youngest cells, either in connective tissue (Figs. 24—27) or in blood (Figs. 20—23), the nucleus presents no difference from any ordinary leucocyte-nucleus; the chromatin is arranged in lumps and threads as usual; if anything, the spaces between the threads of the nuclear network are rather larger than usual. As the nucleus becomes older the nuclear reticulum becomes closer and contains relatively more chromatin (Fig. 40); if it passes into amœboid movement or becomes older still (*i.e.* the cell becomes larger) the staining with methylene-blue becomes more and more diffuse until at last no structure can be made out (Figs. 28, 30, 33). On analysing this with the Ehrlich-Biondi stain, one finds that this diffuseness of staining is due to the presence of large masses of "basichromatin," staining green, while the amount of pink-stained material, whether linin or "oxychromatin" is very small, and no pink nucleoli are to be seen. I have drawn several such nuclei in Figs. 34—37, and have placed beside them as a contrast the nucleus of a young connective tissue cell (Fig. 38), from the same preparation, which shows well-marked nucleoli and a much more open arrangement of the

reticulum. Reinke[94] has found mitosis once, but only once, in basophile cells. I myself have never been fortunate enough to see it. This extreme rarity of mitosis, taken along with the enormous number of basophile cells in the body, and the relative frequency of degenerated forms, goes far to disprove Kanthack and Hardy's contention that the coarsely granular basophile cells form an isolated group, and to render it almost self-evident that they must be derived from the smaller cells of the same kind, and that these again must be derived from the lymphocytes.

I have not been very successful in staining the centrosomes of the basophile cells, and it is only in the smaller forms, such as those in leucæmic blood and in the mesentery of the rabbit, that I have been able to feel certain about them. The ordinary methods are hardly applicable. I have tried staining pieces of mesentery with iron-hæmatoxylin, but unfortunately the basophile granules do not retain the stain, so that it is practically impossible to say whether the centrosomes one does see belong to basophiles or not. The thickness of the piece of tissue also interferes greatly with proper differentiation. If one tries an after-stain of methylene-blue, it is only to find that the iron-hæmatoxylin has destroyed the metachromatic reaction, and that everything, including the parts which have retained the iron stain, has taken a uniform blue colour. The difficulty is the same if sections of the mesentery or of other tissues are used, and there is the additional difficulty that a very small part of a basophile cell is seen in each section. Heidenhain has discussed the relation of the centrosomes to basic dyes, and has come to the conclusion that they have no very special affinity. He admits that they do stain with some basic dyes; Van der Stricht, Hermann, and Flemming have stained them with saffranin and gentian-violet, but he himself had negative results both with these and with methyl-violet, methyl-green and thionin. He does not mention methylene-blue. With this they certainly do stain, not in such a way as to constitute a specific reaction, but well enough to allow one, in favourable cases, to make out the relations. Figs. 23 and 24 show the centrosomes, one in a leucocyte from the blood, the other from the mesentery of the rabbit. Neither cell presents any difference of morphological characters from what one might see in an eosinophile cell; change the colours, and no one would know the difference. The distinction in staining between the centrosome and the granules of the cell is slight but distinct; the granules are blue-green, while the centrosomes are rather of an apple-green, of very nearly the same shade of colour as that which the

hæmoglobin in the red corpuscles assumes when treated with methylene-blue.

In the large basophile cells I have not been able with certainty to make out the position of the microcentre. A frequent appearance is that represented in Fig. 43, where a single large granule forms a centre for a number of smaller ones.

It is of course in the cell-body and in its granules that the main interest of the basophile cells centres. Here, just as in the acidophile group, we find that the granules merely form part of the mitoma, that they are altered microsomes.

I shall best be able to describe the changes through which they pass by tracing the cells from the original lymphocyte or hyaline transition-form, from which they are derived. The first change consists not so much in an enlargement of the microsomes as in the superior visibility of the mitoma. As in the eosinophile cells the cell-body becomes larger, and as there the mitoma takes up acid stains, so here it takes up basic stains (Fig. 4). As the cell increases in size, the mitoma retains its visibility and the microsomes become larger and assume the ordinary appearance of granules. These are at first few and scattered irregularly through the cell: some microsomes have increased in size, others have not; the mitoma is always most visible near the microsomes which have taken up the stain. At this stage, illustrated by Figs. 21—23, from the blood, and Figs. 24—27, from the mesentery, the granules always differ markedly in size, much more so than at later stages. They are always uniform in staining, and curiously enough, the very small ones do not often show the metachromatic reaction with methylene-blue. The cells in the blood are generally spherical, but those in connective tissue soon show an irregularity in shape suggestive of amœboid movements; they put forth those long flat processes which are so characteristic of the larger cells. In Figs. 26 and 27, for instance, all the granules to be seen in the cell are shown, and they were all lying almost in one plane, so that hardly any focussing was required to see them. They must therefore have been lying in a very thin layer of protoplasm, and from the constancy of this appearance we may infer that the spaces through which these leucocytes have to pass between the planes of connective tissue in the mesentery must be very shallow. The rounder cells are generally found in places where, as near the larger blood vessels, the mesentery becomes thicker and allows more space; the elongated cells are generally in the thin parts of the structure.

As the cells become larger the granules, as a rule, but not invariably, also grow larger, and become, generally speaking, more uniform in size. I have never seen a cell, however, in which all the granules were quite uniform, though many authors postulate this for all large basophile cells. In all the drawings I have given I have taken the greatest care not only to put the granules in the exact position in which I saw them, but to represent their relative size, and in Figs. 20—32, 41, 42, 44 and 45 (the two latter being degenerated basophiles) I have tried as far as possible to give their colour also. For while it is in these large cells that the most marked metachromatism is to be found, it is to be noted, that in very few cells are the granules stained uniformly red. Some of the very large cells in the rat's mesentery form an exception, but it is more usual to find some granules bright red, others blue, and to find between these all shades of purple. Still the prevailing effect is red.

In these large cells too, the mitoma is specially easy to make out, and particularly in those which are apparently in amœboid movement or which are very much branched. It is a little difficult to define accurately the colour of which it stains. In the small cells there is no doubt about its being blue, but in the large metachromatic ones it is not so often red as a faint purple.

It has often been remarked that the large cells show a great tendency to leave their granules behind them, thus one might come on a group of granules while the nearest cell was far away. Ballowitz[3] was, I think, the first to declare that all or most of these groups of granules were attached to the cell by fine protoplasmic bridges. It is not indeed always easy to show this; in the cell shown in Fig. 31, which was so isolated that there could be no doubt that all the granules represented belonged to it, no trace could be made out of threads extending from granule to granule. They were probably stretched too much to allow them to be visible. Fig. 22, however, taken from the rat also, but not from the same individual, shows the mitoma very clearly, probably because the sheet of cell-substance has not been so greatly stretched. In the elongated forms one often sees narrow bridges, like those shown in Figs. 29, 30, 33, 41, which connect two larger masses of the cell-body; these often consist of a row of granules with a single thread running between (Figs. 29 and 42). As I shall point out when discussing the degeneration of these cells, I have little doubt that when that stage is reached these bridges are torn across, and the granules are actually left behind. This forms an exact parallel to what happens in the eosinophiles of the newt's blood.

I have spoken as if the elongated basophile cells extended in one plane only, but though this is the case in the membranes, it is not so when these cells occur in ordinary connective tissue. In the heart of the frog or newt, for instance, the branches run in every direction. Fig. 33 will give some slight idea of this.

The staining reactions of the granules and mitoma are always different in degree and often different in kind from those of the nuclear chromatin. With methylene-blue for instance the nucleus of the cell always stains blue; not only the basichromatin, but the oxychromatin, the nucleoli (if they are present) and apparently the linin also retain that colour. The cytomitoma and its microsomes or granules may become, with the same stain, blue of a lighter shade than the nucleus, greenish-blue, red, or all shades of purple. With methyl-green, the most accurate reagent for chromatin inside the nucleus which we possess, the mitoma and granules never stain very satisfactorily, when the stain is allowed to act selectively. The basophile cells which stain best with methyl-green are I think those of the frog, but the colour is always much fainter than in the nucleus, so faint that it is often difficult to pick out the cells. Neither the small cells of the blood nor the large ones of the newt's connective tissue stain with methyl-green at all. The latter are of course markedly metachromatic. Westphal[4] noticed that a methyl-green solution in which methyl-violet was present as an impurity stained the granules violet. With the ordinary basic stains, as dahlia, gentian-violet, basic fuchsin, &c. the difference when the dyes are used directly is mainly one of degree, but the fact that when acetic acid is used to differentiate, the granules retain the stain while the nuclei lose it, shows that the difference must be one of kind.

The interstitial substance is not basophile.

As the basophile cells degenerate the change of which I have already spoken as taking place in their nuclei as they become larger (*i.e.* older), continues and increases. With methylene-blue they stain very deeply, and absolutely homogeneously, and even with the Ehrlich-Biondi stain hardly a vestige of structure can be made out. The nuclei retain or resume their rounded shape.

It is in the cell-body of the elongated and ramified forms however that the greatest change occurs. It will have been noticed that the tendency is for one of these long cells to consist of a row of islets of protoplasm connected by delicate bridges. As long as the cell is obviously living one can generally trace the connection between these islets, but as the cell degenerates it seems to die first at its extremities

and the bridges are broken through, leaving masses of granules lying free in the tissues. The further process in these is similar to that in the detached portions of the eosinophiles in the newt's blood.

In the portion of cell-body which remains round the nucleus the granules are generally large; the threads of the mitoma lose their power of taking up stains, and the cell assumes the form of a more or less disc-shaped mass of granules, which not only stain badly, but which lose their regular outline. It is curious that in all the markedly degenerated basophile cells which I have seen the granules stained blue with methylene-blue and seemed to have lost the red metachromatism. In Fig. 45 this is well seen. Some of the granules in Fig. 44 retain the red colour to a certain extent, but the drawing rather exaggerates the amount of red in them.

The significance of the enlarged microsomes or "granules."

Ehrlich[4] regarded the seven varieties of granules which he described as being all formed by the cells, and as being either reserve material or products for excretion. Hankin[9] took the view that the acidophile granules were secretory products, containing "alexines," and destined to be secreted into the blood or lymph. Kanthack, Hardy, and Keng [11, 14, 22] have taken much the same view of these special granules. Sherrington[25] has thrown doubt upon it, and Metschnikoff[26] disputes it, and regards the eosinophile granules as reserve material.

All these authors regard the granules as paraplastic. M. Heidenhain[19] surmised that the "neutrophile" granules at least might be protoplasmic and merely special states of the cell-microsomes, but did not follow up his hint. It is evident from the foregoing pages that the granules are plasmatic and not paraplastic, and it is therefore exceedingly unlikely that they are products for excretion. As regards the question whether they are reserve material, it is hardly likely that the more reserve material a cell accumulates the more rapidly it is able to move. *A priori* one would expect that reserve material would be carried in a paraplastic form, as Ehrlich and Metschnikoff of course supposed it to be in the eosinophile cells, and as it is stored in most cells which we know to accumulate reserve material. If the granules consisted of reserve material one would expect them to disappear or alter when the cell was placed in unfavourable circumstances, but these cells are those which survive in pus with least change in either cells or granules.

My own opinion is that this altered condition of the microsomes, in the acidophile series at least, has to do in some way with the increased power of amœboid movement of the cell. It is certainly interesting to note that the more active the cells of this series become, the more visible become their mitoma and the microsomes which form part of it. The lymphocytes in which no mitoma can be seen are practically non-amœboid. The "hyaline" cells in which it is not very evident move but sluggishly. The oxyphile cells, with a well-marked mitoma and microsomes, move more rapidly, and the eosinophile cells, whose mitoma and microsomes are the most visible of all, move most rapidly.

Whether it is the threads of the mitoma or the microsomes which are the more active agents in movement it would be difficult to say. It is certain that the length of thread lying between the microsomes varies immensely in different parts of the cell and the short threads are usually the more deeply stained, so that it looks as though they were contracted and therefore thickened. On the other hand the microsomes at the periphery are, generally speaking, the largest, and there can be no doubt that it is the circumference of the cell which moves most and moves furthest. The motion must be smallest at or about the microcentre, which is the point of insertion of all the threads. On the whole I am inclined to believe that the microsomes are not simply dragged about by the mitoma, but that they have themselves an active share in its movements.

I do not know whether to regard the basophile cells in the same light as the eosinophile cells or not. Our data as to their movements and functions are altogether too scanty to allow as yet of any definite opinion, but as far as one can judge from fixed specimens the larger basophile cells seem to have more power of movement than the smaller ones.

The physical relations of nucleus and cell-body in leucocytes.

M. Heidenhain [23] has recently described the cell-substance of leucocytes as containing a small group of centrosomes—the microcentre—and of threads radiating from them to the periphery of the cell. He considers that the nucleus lies free in the interfilar spaces, and is not organically connected with the cell-substance, and he has advanced a theory as to the relative action of the centrosomes and of these threads—or "organic radii"—to explain the movements of the cell and the changes in shape of the nucleus. While I can confirm almost everything he has put forward as a statement of fact in regard to the relative position of

nucleus and centrosomes, I differ from his view of the cell-structure, in so far as I find that the threads or "organic radii" are always connected by cross-bars running between the microsomes, so that the whole forms a network, which is undoubtedly centred in the group of centrosomes. Nor can I believe that the nucleus lies free in the interfilar spaces.

The theory can in the main be accepted, for it explains much in leucocyte structure which was hitherto unintelligible, and some of the principal difficulties in the theory can be overcome by assuming a connection between the network of linin in the nucleus and the network of the cell-substance, not by means of the organic radii, but by the cross-bars which unite them.

If this be the case, the nucleus by its increase in size at the end of mitosis would still push the radii aside as they leave the microcentre, and thus remove one of Heidenhain's reasons for believing that the nucleus is free in the interfilar spaces. The microcentre would be able to pass to any necessary distance from the nucleus, in the amœboid movement of the cell, for we may postulate for the connecting strands the same contractility and extensibility which the radii themselves have. In showing that the mitotic spindle is derived from the centrodesmosis (the band uniting the centrosomes in the microcentre), Heidenhain has shown how great the extensibility of such structures is. The power of rearrangement and adaptation to circumstances in the cytomitoma must be enormous, as the budding of each new centrosome must involve the splitting into two of each of the organic radii, and in the comparatively rapid progress of mitosis the whole cell-network is entirely rearranged.

Heidenhain is too anxious to prove that the nucleus is independent of the cytomitoma, and in order to do this he regards it first as a soft body, in order that it may be compressed by the organic radii, and then as a rigid body, in order that any threads connecting it with the cytomitoma might be shown to be broken if any strain were put upon them. We have nothing to show us that there is any essential difference, either chemical or morphological, between the extranuclear and the intranuclear linin network. It seems possible that the intranuclear linin threads, many of which do not contain microsomes of chromatin, may yield and stretch or even be pulled out of the nucleus, as thread is pulled off a reel, when strain is put upon them from the outside.

It is only on the hypothesis that the linin network of the nucleus

has the power of movement that the infinite variety of arrangement of the chromatin in leucocyte-nuclei can be explained. The nuclei of the lymphocytes, the products of mitosis, resemble one another so closely as to be indistinguishable. At the ends of the series, and in the intermediate stages, there are differences not only in the shape of the nucleus, but in the arrangement of the chromatin within it, such as we find in no other set of cells. The tendency at present is to regard the chromatin as passive; the view that the centrosomes are merely points of insertion for the mitoma is gaining ground over the theory that they control the movements of the cell, and we are consequently forced to consider that the linin network which runs through both cell-body and nucleus is, if not certainly the source of movement, at any rate the means by which movement is carried out.

We see that the linin outside the nucleus moves, we know that the nucleus as a whole changes its shape, why should the linin within the nucleus not move also? It is obvious that its movements are at any rate co-ordinated with those of the cell-body, or we might have in an amœboid cell the spectacle of the nucleus and the cell-body pulling in different directions. It is hard to fancy that this co-ordination can take place without such an actual physical connection as I have described.

An interesting corroboration of the general truth of Heidenhain's contentions is to be found in the large leucocytes of the mussel. These are nearly all eosinophile, and are very actively amœboid. In spite of this it is extremely rare to see one of the nuclei of these cells assume any greater modification of the spherical form than a slightly oval shape, and the meaning of this evidently is, that the nucleus is very small in proportion to the cell-body, that it has practically always been driven to the periphery of the cell, and that there it escapes the distorting action of the movements of the microcentre.

Summary.

Leucocytes whose finer structure is to be examined must be fixed by reagents, of which the best is sublimate; drying is unreliable, as it does not preserve details of structure.

It is impossible to divide leucocytes into a hæmal variety and a cœlomic variety, for (1) lymphocytes are the precursors of all forms; (2) leucocytes are not present in the blood in early fœtal life; (3) they are constantly passing from blood to connective tissue and *vice versâ*;

(4) the mitotic reproduction of leucocytes takes place almost entirely in adenoid tissue. It is only their size which prevents the largest hyaline, eosinophile, and basophile cells from appearing in the blood.

M. Heidenhain's observations on the relative positions of nucleus and centrosomes are correct. The "organic radii" are not entirely separate from one another, but are connected at every microsome by subsidiary threads. The nucleus does not lie free in the interfilar spaces, but its linin network is connected with the cytomitoma, and the two are perhaps to a certain extent interchangeable. This would explain the variety of arrangement of the nuclear chromatin, and the co-ordination in movement of the cell-body and nucleus.

The shape of the nucleus has no relation to the presence or absence of granules in the cell-body, but depends on (1) the relative sizes of the cell-body and nucleus (according to M. Heidenhain's law); (2) the position of the centrosomes; (3) the condition of rest or movement of the cell.

All varieties of leucocytes are merely stages in the development of a tissue. They may be divided for convenience, and with regard to the presence or absence of granules, into three main groups, the Hyaline, Acidophile, and Basophile. These forms are all derived from the lymphocytes, which are the daughter-cells derived from the mitosis of all leucocytes (except the largest granular forms). From the lymphocytes or from the small hyaline forms the three groups branch off.

I. The cells may remain *Hyaline*, and attain their maximum development in such cells as the large phagocytes of the alimentary canal or the giant-cells of bone-marrow. In this case they remain sluggishly amœboid, their centrosomes are very evident, and the cytomitoma and microsomes, while visible, are not easily made out.

II. In the *Acidophile* group are included the so-called neutrophile and amphophile cells, which are really oxyphile, and the eosinophile or coarsely granular cells. In the transition-forms from the small hyaline cells to the oxyphiles the mitoma of the cell-body becomes more visible, and it is this rather than the presence of granules which gives rise at first to the finely granular appearance. The granules are simply the microsomes of the mitoma. In the transition-forms between the oxyphile and eosinophile cells some of the microsomes become larger and take up acid dyes and iron-hæmatoxylin better than others, and when the large eosinophiles are reached the threads of the mitoma have become thicker, and also take up iron-hæmatoxylin. The microsomes in the eosinophiles vary greatly in size; they are arranged

subradially, with the largest microsomes usually at the periphery. In this group the centrosomes are well seen, better in the eosinophiles than in the oxyphiles.

III. The cells of the *Basophile* group vary immensely in size and shape. The nucleus is generally ellipsoidal, except when the cells are actively amœboid, probably because the cell-body is usually relatively large. As the cells increase in size the amount of basi-chromatin in the nucleus becomes increased out of proportion to the size of the nucleus. This has no relation to mitosis, which has only once been observed in these cells. The centrosomes can be seen fairly easily in the smaller rounded forms. In the larger forms they are difficult to make out, and it seems possible that there may be several subordinate centrosomes. These cells are derived from the small hyaline forms by an increased visibility of the mitoma, and an increase in size of the microsomes, which are at first usually very irregular in size, and do not exhibit metachromasia with methylene-blue. As the cells become larger, so generally do the granules; they become more uniform, but are never all quite of one size. Metachromasia becomes more constant, and the mitoma more evident, especially in those cells which seem to be moving, or which are greatly branched. The staining of the microsomes is never quite the same as that of the nuclear chromatin.

The granules of leucocytes are therefore *not* products of the metabolic activity of the cell embedded in a structureless protoplasm, as was hitherto supposed, but represent an altered condition of the microsomes. They always form part of the cytomitoma, and are therefore plasmatic, and not paraplastic. They are probably concerned with amœboid movement, and they and the rest of the mitoma are more visible the more active the cell.

In diseased conditions it is probably impossible to say what organ is affected from the kind of leucocytes present in excess in the blood.

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DESCRIPTION OF THE FIGURES.

The objective used in all cases except Fig. 41 was Zeiss's apochromatic homogeneous immersion 2·0 mm. equivalent focus and 1·40 mm. aperture, and the eyepiece was 18 compensating, in all cases but Figs. 3, 6 and 41. Figs. 3 and 6 were drawn with eyepiece 8 compensating. Fig. 41 with eyepiece 2 compensating and Zeiss objective *DD*.

The magnification for the majority of the figures is × 2250; for Figs. 3 and 6 × 1,000, for Fig. 41, about × 200.

Fig. 1. Hyaline leucocyte from blood of newt, moving slightly, astrosphere very evident. Iron-hæmatoxylin.

Fig. 2. Small oxyphile leucocyte from newt's blood, mitoma very evident, nucleus rather over-stained. Iron-hæmatoxylin.

Fig. 3. Eosinophile cell from newt's blood with spherical pseudopod. *n* = nucleus, which was over-stained. Iron-hæmatoxylin, × 1,000.

Fig. 4. Basophile cell, very small, from rabbit's mesentery. *n* = nucleus, *c* = centrosomes nearly equal in size, the centrodesmosis took up the stain only *very* faintly. Methylene-blue.

Fig. 5. Detached pseudopod from eosinophile cell of newt's blood; the optical section of the middle is drawn; the points unattached represent the places where the mitoma passed out of view, either upwards or downwards. Methylene-blue, Bordeaux.

Fig. 6. Eosinophile cell of newt's blood with spherical pseudopod attached by thin thread. *n* = nucleus over-stained. Bordeaux, iron-hæmatoxylin.

Fig. 7. Hyaline leucocyte of frog's blood. *as* = astrosphere with two centrosomes very unequal in size; some of the microsomes in the ring bounding the astrosphere have retained the stain; *n* = nucleus over-stained. The cytomitoma though visible was not evident enough to be drawn in detail. Bordeaux, iron-hæmatoxylin.

Fig. 8. Oxyphile leucocyte (amphophile) from bone-marrow of rabbit; mitoma very evident, microsomes small. Iron-hæmatoxylin.

Fig. 9. Hyaline leucocyte from bone-marrow of rabbit; microcentre with four centrosomes, mitoma fairly evident. Iron-hæmatoxylin.

Fig. 10. Eosinophile cell from bone-marrow of rabbit; astrosphere well seen, two small centrosomes. Iron-hæmatoxylin.

Fig. 11. Transition form between oxyphile and eosinophile cells from bone-marrow of rabbit, evidently very small part of the cell. Iron-hæmatoxylin.

Fig. 12. Eosinophile cell from bone-marrow of rabbit; astrosphere well seen. The section has evidently passed right through the middle of the cell, and through the middle of a spectacle-shaped nucleus. Iron-hæmatoxylin.

Fig. 13. Part of an eosinophile cell from bone-marrow of rabbit; the fainter microsomes were those slightly out of focus, above or below, when drawing the nucleus. Iron-hæmatoxylin.

Fig. 14. Eosinophile cell from tongue of frog; fainter microsomes slightly out of focus. Iron-hæmatoxylin.

Fig. 15. Part of an eosinophile cell from tongue of a frog; no nucleus in the section, mitoma specially well seen. Iron-hæmatoxylin.

Fig. 16. Eosinophile cell from hen's ovary; astrosphere well seen; radial (?) arrangement of microsomes. Section probably cut as in Fig. 12. Iron-hæmatoxylin.

Fig. 17. Hyaline leucocyte from newt's blood which has ingested the detached pseudopod of an eosinophile cell. Ehrlich-Biondi.

Fig. 18. Oxyphile cell from human blood, with polymorphous nucleus, microcentres in middle of cell. Ehrlich-Biondi.

Fig. 19. As 18, rather larger cell.

Figs. 20—22. Small basophile leucocytes from human blood (leucocythæmia). Methylene-blue.

Fig. 23. The same, but showing the microcentre.

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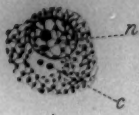

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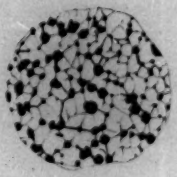

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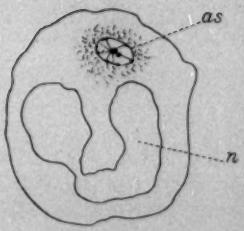

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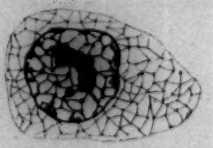

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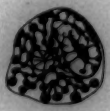

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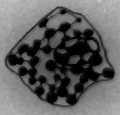

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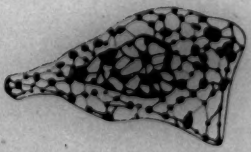

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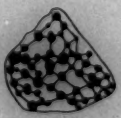

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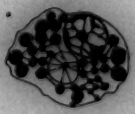

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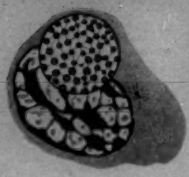

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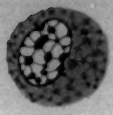

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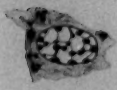

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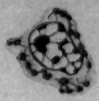

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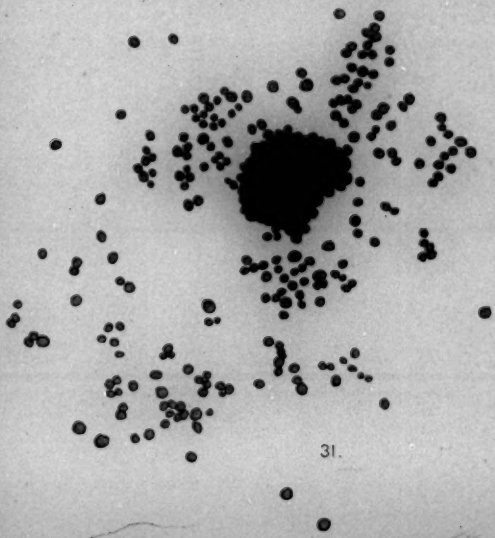

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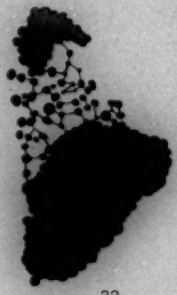

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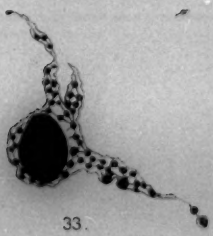

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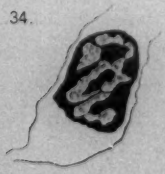

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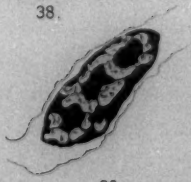

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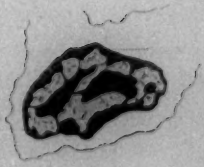

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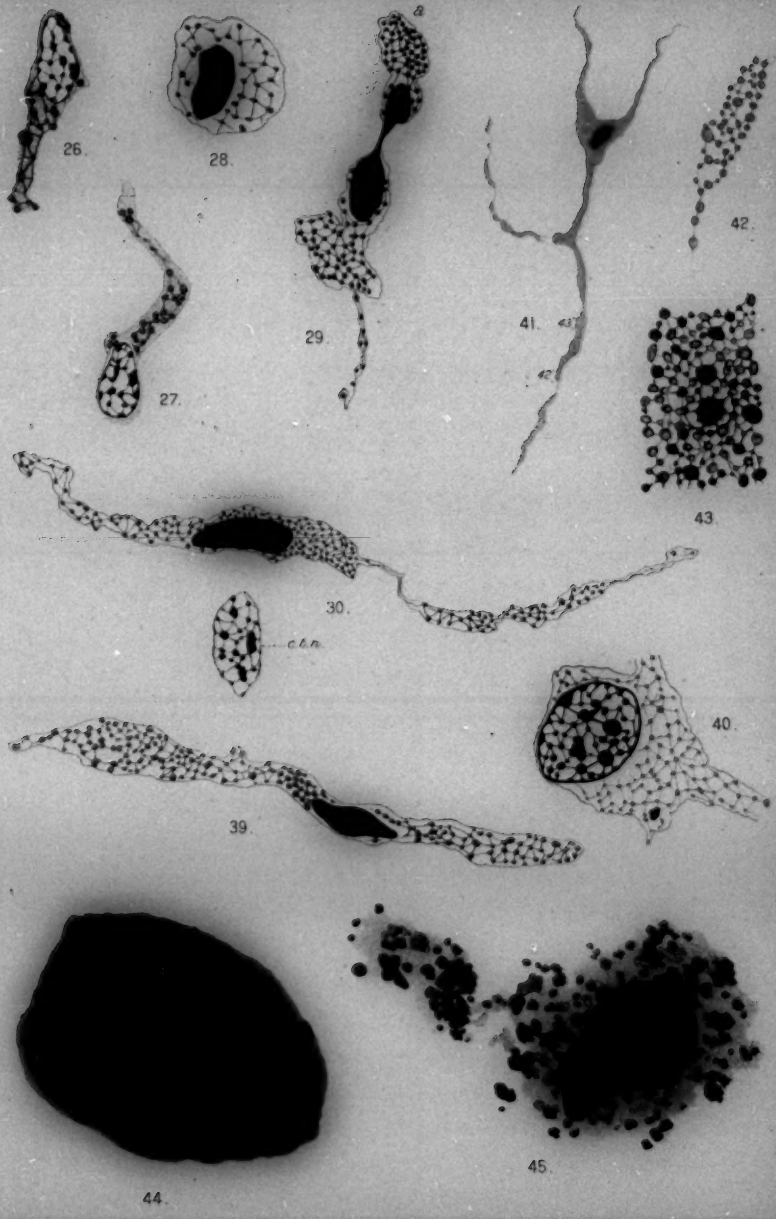


Edwin Wilson, Cambridge.

ROYAL
SOCIETY

Figs. 24—27. Small basophile cells from the mesentery of an adult rabbit. Methylene-blue.

Fig. 28. Round basophile cell from mesentery of guinea-pig. Nucleus deeply stained. Methylene-blue.

Fig. 29. Amœboid basophile cell from mesentery of guinea-pig. The cell is passing through a narrow opening. *a* = anterior end. Methylene-blue.

Fig. 30. The same. *c.t.n.* = nucleus of one of the connective tissue cells, across which the basophile cell is passing, to show the difference in character of the nucleus.

Fig. 31. Large flat basophile cell from mesentery of rat. Methylene-blue.

Fig. 32. The same, mitoma more evident.

Fig. 33. Section through a basophile cell in the heart-wall of the newt; nucleus deeply stained. Methylene-blue.

Figs. 34—37. Nuclei of basophile cells from peritoneum of newt; granules omitted. Ehrlich-Biondi.

Fig. 38. Nucleus of connective tissue cell from peritoneum of newt. Ehrlich-Biondi.

Fig. 39. Section of basophile cell from heart-wall of newt. Nucleus deeply stained. Methylene-blue.

Fig. 40. Nucleus and part of cell-body of a young basophile cell from peritoneum of newt. Methylene-blue.

Fig. 41. Basophile cell from peritoneum of newt. Methylene-blue, × 200.

Fig. 42. Mitoma from part of Fig. 41, marked 42.

Fig. 43. Mitoma from part of Fig. 41, marked 43.

Fig. 44. Degenerated basophile cell from mesentery of newt. Methylene-blue.

Fig. 45. The same; a later stage.

CONTRIBUTION TO THE STUDY OF LYMPH-FORMATION WITH ESPECIAL REFERENCE TO THE PARTS PLAYED BY OSMOSIS AND FILTRATION.

By W. S. LAZARUS-BARLOW, M.D., M.R.C.P., *Demonstrator of Pathology in the University of Cambridge.* (Thirteen Figures in Text.)

(*From the Pathological Laboratory, Cambridge.*)

Introductory.

In a previous paper[1] I pointed out that before the laws of osmosis deduced from the final osmotic pressure, freezing point, etc. could be applied to the explanation of biological problems it was necessary to determine whether the initial rates of osmosis of substances bear constant ratios to their final osmotic pressures, and whether the presence of proteid substances in the solutions affects the initial rate of osmosis. I found that the initial rates of osmosis cannot be determined from observations of the freezing points of solutions, *i.e.* from their final osmotic pressures, and that proteid substances even when present only in minute quantities markedly diminish the rate of osmosis. In the present paper the conclusions thus obtained have been applied to the consideration of lymph-formation, and data bearing upon the question have in addition been derived from observation of the specific gravity of blood, of voluntary muscle and of lymph as well as from those factors (amount of lymph flow, etc.) which have hitherto been especially investigated in this connection.

Section I. Methods.

Throughout the research dogs were used; they were weighed carefully with the bladder empty and their weights varied between 7 kilos. and 24·5 kilos. They were not given solid food for 24 hours before an experiment but were allowed as much water as they required. Upon this latter point great stress was laid, since it had been found that the

[1] This *Journal*, xix. 140. 1895.

actual results obtained in any experiment vary considerably in degree according as water has been allowed or withheld. They received half-an-hour before complete anæsthetisation a hypodermic injection of morphia varying from 1 to 4 grains according to the size of the animal. The further anæsthetic used throughout the research was a mixture of equal parts of chloroform and ether. Special care was given to the administration of the anæsthetic. By reason of the immediate effect of an anæsthetic upon the lymph flow a small quantity was given about ten minutes before an injection into the veins took place, and for five minutes before the injection until slight evidence of movement on the part of the animal occurred—a period frequently of an hour if a sufficient dose of morphia had been given before commencing the experiment —no additional anæsthetic was administered, or if any were necessary only sufficient was given to prevent movements of the animal. Of course it is obvious that for the obtaining of satisfactory data, complete anæsthesia and therefore complete relaxation of muscles are necessary, but it is equally important that no rapid modifications of the degree of anæsthesia should be produced.

The experiment was commenced by introducing a cannula into the thoracic duct, the lymphatics from the head and neck on the left side being ligatured as far as possible. Next the left external jugular vein was dissected out and a cannula provided with a tap inserted into it. After the right carotid had been connected with a mercury manometer, the femoral artery or one of its branches close to the main vessel was dissected out and so arranged that small quantities of blood could be removed at intervals for the estimation of its specific gravity. In those cases where the venous pressure was measured the femoral vein was ligatured below and a long cannula inserted upwards, so as to obtain the pressure as near to the inferior vena cava as possible: as a matter of fact, dissection after finishing the experiment generally showed that the cannula terminated internally in the common iliac vein. The outer end of the cannula was connected with a manometer filled with saturated magnesium sulphate solution. If the pressure in the portal vein was to be estimated (and this was done only in a minority of cases) an incision was made on the left side of the abdomen, the spleen drawn out, the arteries supplying it ligatured, and a cannula pushed well into the splenic vein. It is now well recognised that opening of the abdominal cavity alone produces marked modifications of the specific gravity of the blood[1] and of the tissues, and therefore the severe procedure,

[1] Sherrington. This *Journal*, XIV. 83. 1893.

necessary to obtain the portal pressure was considered to introduce sources of error more than counterbalancing any advantage that might accrue from its estimation. For the same reason throughout the whole investigation the kidneys were not interfered with; apart from the actual effects produced by the operation, removal of these organs, which are essentially the regulating mechanism for the composition of the blood, from the circulation, introduces so much general disturbance of the animal economy, that it was thought better to leave them untouched; though no doubt the results of injection are diminished by the removal of the crystalloid from the blood, and further complicated in all probability by differences in the rates at which the various substances are excreted.

The specific gravity of the blood and that of the lymph were taken by Roy's method. With regard to the specific gravity of the tissues, voluntary muscle (Sartorius and Rectus abdominis) was used as in a research upon the pathology of the œdema which accompanies passive congestion[1], but the method employed in the present research was different and more satisfactory in many respects. I have used here solutions of gum arabic in water made up to specific gravity corresponding to the even numbers between 1054 and 1074, both inclusive. These solutions were kept in tightly stoppered bottles and to each alternate bottle a minute trace of solid methylene blue was added. Now since diffusion between gum solutions takes place with great slowness, it is possible to place in a large test-tube portions of the gum-solutions in the inverse order of their densities, and if care be taken not to mix them when introducing them into the test-tube, a column is formed consisting of alternating blue and white bands, each band corresponding to a different specific gravity. Such a column of solutions will quite well show the different layers after a period of 24 hours if left undisturbed, though the edges of the layers will not be so clearly defined as at first. The accompanying figure shows diagrammatically the appearance of such a column of fluid ready for an experiment (Fig. 1).

Fig. 1. Diagrammatic appearance of column of gum solutions for estimating specific gravity of solid tissues.

For the estimation of the specific gravity of a muscle by this method, one, or at most two pieces of the tissue are necessary, since it

[1] *Phil. Trans. Roy. Soc.* 1894, B, p. 783.

will fall through those layers which are of lower specific gravity than itself and will come to rest in that layer with which its own specific gravity most closely corresponds. By this method it is possible to take a considerable number of estimations at different times from the same muscle, an obvious advantage, since different muscles are of different specific gravity. A column of fluid 25 mm. in cross-section can be used for ten or twelve different estimations during an experiment lasting six or eight hours. Further details need not be given here, since the process and method is described fully elsewhere[1].

Method of estimating the initial rate of osmosis of blood-serum and of lymph.

Since it has already been shown[2] that the freezing point method is unreliable so far as the determination of osmosis in the animal body is concerned, the initial rate of osmosis[3] of samples of blood-serum and of lymph have been directly estimated in the following way. An osmometer was made suitable for working with the small amounts of fluid at times at our disposal. This instrument was designed to determine the initial rates of osmosis of samples of blood-serum and of lymph and therefore like the larger instrument described elsewhere was arranged horizontally; but from its small size it was unnecessary to provide it with a support for the membrane while equality of pressure on the two sides of the membrane was obtained in a different manner. The instrument is shown in the following diagram.

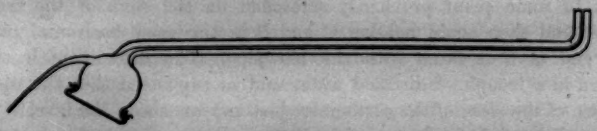


FIG. 2.

Osmometer for estimating the initial rates of osmosis of small quantities of fluids. For description see text.

It is made of glass throughout, and its length from D to x is 100 mm. The bulb A holds approximately 1 c.c. B is formed of thermometric

[1] *Proc. Camb. Philosoph. Soc.* 1895. Vol. VIII. Pt. 5, p. 279.

[2] Lazarus-Barlow. This *Journal*, *loc. cit.*

[3] By the term "initial rate of osmosis" is here meant the numerical value which represents the rate at which any solution of a crystalloid attracts water through a membrane at atmospheric pressure, compared with the rate at which decinormal glucose solution attracts water under identical conditions. Glucose was taken as the standard because of its comparatively low rate of dialysis.

tubing having an internal bore of 1·5 mm. At x it is bent upwards at a right angle, care being taken not to constrict the bore. At D the thermometric tube expands into the bulb A, the main axis of which is inclined to B at an angle of about 45°. Into the bulb is inserted a side-piece C, the essential points of which are, firstly, that it should at its distal end be of capillary dimensions so that it may readily be fused off, and secondly, that it should be inclined at such an angle (approximately parallel to the main axis of the bulb) that when filling the instrument through it, the mouth of the bulb carrying the membrane may be horizontal. If this be not done fluid may pass into the thermometric stem while air still remains in the bulb. Apart from the mere mechanical disadvantage thus introduced, in many cases the amount of fluid (especially lymph) at our disposal for an estimation may be so small that all risk of losing any of it must be avoided. By having C inclined this difficulty is entirely obviated. The membrane used was prepared peritoneal membrane of calf, and it was attached wet over a collar of india-rubber, behind the projecting lip of the bulb, by means of a few turns of waxed silk.

The instrument is filled by dipping the open end of C beneath the fluid to be investigated and exhausting with the mouth through the open end of the thermometric bore. When the bulb is full and a certain amount of fluid stands in the thermometric stem the instrument is turned in such a way that C and B are horizontal. By slightly depressing the end C the free edge of the fluid is brought back to some point previously scratched on the stem of the osmometer and then again holding C and B in the same horizontal plane the end of C is fused with the blowpipe. The instrument is then placed in a trough of distilled water and so supported that the upper surface of the stem of the osmometer just appears above the level of the water, the upright limb at x of course standing well out of the water: the levels of the fluids inside the osmometer and outside being the same, it follows that the membrane is exposed to no hydrostatic pressure. Beneath the stem of the instrument is placed a millimetre scale and the effect of immersion in the water of the trough upon the position of the edge of the fluid in the stem is immediately noted. Through the membrane water passes by osmosis, and the distances which the free edge of the fluid travels in an hour in the case of different fluids indicates the relative initial rates of osmosis of these fluids.

By using the same membrane and instrument throughout a series of estimations comparative results can be obtained, but since after a

long experiment the initial rates of osmosis of as many as 70 different fluids have sometimes to be investigated, each for an hour, the length of time occupied between the first and last estimations would be so great that putrefactive and other changes would seriously interfere with the accuracy of the estimations. Six osmometers, therefore, made after the same pattern as the one described, but not absolutely alike in every respect, were actually used and the results corrected in the following way. All the instruments were filled to the zero-point with decinormal glucose solution, fixed on the stand to be described immediately, and at the same instant lowered into the trough of distilled water. The position of the free edge of the liquid on the millimetre scale was immediately noted and the instruments were left undisturbed for an hour, when readings were again taken. The instruments having been filled with the same solution and the conditions under which osmosis was allowed to take place being absolutely identical in all of them, it follows that the distances travelled by the edges of the fluids in the stems of the osmometers gave data whereby results obtained with any one instrument could be expressed in terms of any other. Supposing, for example, the distance travelled by the edge of the glucose solution in the stem of osmometer I = A, and in the stem of osmometer II = B, then if lymph under similar circumstances travelled a and b in each instrument respectively we can determine the distance that would have been travelled by the lymph in osmometer II had it been placed in osmometer I by the formula $B : A :: b : x$ when $x = \frac{Ab}{B}$. Similar formulæ hold good for any number of osmometers and therefore it became possible to use six osmometers and reduce the results obtained with them to terms of any one of them—a procedure which materially shortened the length of time necessary for the estimations, and therefore the degree of change which blood-serum and lymph could undergo from putrefaction. Until a short time before they were required for examination all the specimens of blood-serum and lymph were kept in an ice-cupboard. The corrected results thus obtained give curves representing the variations in the initial rate of osmosis of the blood-serum and lymph referred to a common standard of decinormal glucose solution.

One point further is necessary. It has been shown elsewhere that as the results of constant use of a membrane, and especially when albuminous solutions are being investigated, there is a gradual diminution in the rate of osmosis of the same substance. After all the specimens of blood-serum and of lymph have been determined it is

therefore essential that the osmometers with their membranes be washed as well as possible without otherwise disturbing them with decinormal glucose solution, and the initial rate of osmosis of decinormal glucose solution again estimated. The figures obtained should bear the same ratios to one another as at the first, though they should all be proportionally diminished. It was practically found in the actual research that the diminution in the initial rate of osmosis of the glucose solution was almost exactly the same in all the osmometers. As the results obtained bear on the method alone and not upon the subject-matter of the paper they may be given here. In one representative example the actual distances travelled by the edge of the glucose solution in the stem of the osmometer before and after estimation of the initial rates of osmosis of the blood-serum and lymph were as follows:—

Osmometer	I	II	III	IV	V	VI
	mm.	mm.	mm.	mm.	mm.	mm.
Before	10·75	23	17	14·5	17	15
After	6	12·5	9	7·5	8	8·5
Ratio	1 : 1·8	1 : 1·8	1 : 1·9	1 : 1·9	1 : 2·1	1 : 1·8

It therefore follows that the line representing the initial rate of osmosis of decinormal glucose solution during the time occupied in investigating the initial rates of osmosis of the various specimens of blood-serum and of lymph was one of gradual descent, and since it happens that in this experiment all the results of whichever kind have been reduced to terms of osmometer VI. it follows that the line representing the initial rate of osmosis of decinormal glucose solution must be drawn between 15 at the commencement and 8·5 at the end. This is simpler than making further corrections in the values of the rates of osmosis of the blood-serum and lymph and causing the glucose solution to maintain in the curve a constant level throughout, by reason of the varying factors by which the values obtained for blood-serum and lymph would have to be multiplied according as they were investigated earlier or later. It further follows from the constancy of the ratio between the rates of osmosis of glucose solution before investigating the albuminous solutions and after, that in the formula given above $x = \frac{Ab}{B}, \frac{A}{B}$ is approximately a constant throughout the whole time during which estimations of the initial rates of osmosis were being made.

For convenience the six osmometers were placed on a flat piece of board provided with a central perpendicular stem and grooved to

such a depth in parallel lines that when the stems of the osmometers were placed in the grooves their thermometric bores were on a level with the surface of the board. The bulbs of the osmometers hung over the edge of this support and the stems were kept fixed in the grooves by two elastic bands; a millimetre scale was placed in the bottom of each groove beneath the stem of the osmometer. When the osmometers had been filled and placed on the wooden support it was perfectly simple to lower the whole board into the trough of distilled water until it was just submerged, and fix it in that position by clamping the central perpendicular stem. This done the position of the free edge of the fluid in the thermometric bore of each osmometer was noted and the whole system left undisturbed for an hour. Readings were then taken again, the support and osmometers lifted out from the water, emptied, and filled with fresh specimens of lymph or of blood-serum as before. The osmometers were always washed out with a small quantity of the fluid to be investigated before definitely filling them, and after the rates of osmosis of a series of six specimens of blood-serum had been estimated, the specimens of lymph corresponding to the same times of removal from the animal were immediately investigated, so that corresponding specimens of lymph and of blood-serum were examined with as nearly identical a condition of the membrane as possible. The specimens were not investigated in any definite chronological order and sometimes the initial rate of osmosis of the blood-serum was investigated before the initial rate of osmosis of the corresponding lymph (as far as time of removal from the body is concerned), sometimes after. These two points in view of certain results obtained are of great importance. It is needless to say that the membranes were not disturbed during the whole series of estimations excepting so far as was inevitable during the actual processes of emptying and refilling the osmometers. The blood-serum was obtained by centrifuging the blood after allowing it to clot and breaking up the clot.

This method has the advantage over Hamburger's method of estimating the tonicity of fluids by means of blood corpuscles by reason of the fact that it allows of comparison between different experiments. Decinormal glucose solution and distilled water are of constant composition, and therefore if they are invariably used as the standards, all results obtained with other fluids are comparable. But blood corpuscles are not of constant composition, and they vary in specific gravity[1] even in the same animal. Therefore though results obtained

[1] Lazarus-Barlow. *Phil. Trans. Roy. Soc., loc. cit.* p. 789 note.

when only one specimen of blood is used for obtaining the corpuscles are *inter se* comparable, no comparison except in the broadest possible way can be made between results obtained when another specimen of corpuscles is used for the indicator.

But there is a further difference between the method described and Hamburger's method. In Hamburger's method because hæmoglobin leaves the corpuscles in a certain strength of salt solution and in a certain strength of lymph solution, these two solutions are said to be isotonic; even granting that they are isotonic in the sense that they have the same freezing point it does not follow that they have the same initial rates of osmosis, in fact upon this point no information is given at all, and therefore so far as osmosis is concerned a fallacious idea may be obtained. In other words, Hamburger's method may show that one fluid has a higher "tonicity" than another, but this does not show that a greater amount of water is attracted by the former unless it be also shown that the fluids are composed of identical constituents. This difficulty is avoided by the direct measurement of the initial rate of osmosis as described in the method given above, and when it is shown by this method that of two specimens, of *e.g.* lymph, one attracts more water in a given time than the other there is an end of the matter whatever their compositions may be.

The substances used for injection were glucose, sodium chloride and urea, being those substances whose initial rates of osmosis had already been investigated upon the artificial scheme as described in a former paper. Owing to the expense which would have been incurred in using "chemically pure" substances for these injections in which as much as 80 grams was sometimes introduced into the circulation in a single experiment, the initial rates of osmosis of "pure" glucose, sodium chloride and urea as obtained from the manufacturer were investigated with a view of using them in making up the solutions. It was found that the initial rates of osmosis of these three "pure" substances bear the same relation to one another when peritoneal membrane is used as the membrane throughout, as the "chemically pure" substances, *i.e.* the initial rate of osmosis of "pure" glucose is greater than that of an equimolecular solution of "pure" sodium chloride, and the initial rate of osmosis of "pure" sodium chloride is greater than the initial rate of osmosis of an equimolecular solution of "pure" urea, while the initial rates of osmosis of all three "pure" substances are impeded by the presence of albumen in the fluids, even if that albumen be only

present in very small quantities. When therefore, as for example in an animal experiment, accuracy such as is attainable in a physical experiment is unattainable, "chemically pure" substances are unlikely to give clearer information than "pure" substances, and for this reason the latter and less expensive substances were used.

The solutions were of two kinds, 1st weak, viz. ·75 p.c. sodium chloride solution and 2·25 p.c. glucose solution, and 2nd concentrated, viz. equimolecular solutions of sodium chloride, glucose and urea made up of such strengths (sodium chloride 18 p.c.; glucose 55·38 p.c.; urea 18·46 p.c.) that injection of $\frac{1}{100}$ of the body weight of the animal would add to the blood of the animal (reckoned as $\frac{1}{13}$ of body weight) that amount of the crystalloid as would cause the blood to become, so far as that substance was concerned, a decinormal solution of the crystalloid. An example will make this clearer. Supposing the animal weigh 13 kilos it will contain 1 kilo of blood, and $\frac{13000}{400} = 32·5$ c.c. of each solution severally would be used for an injection. Now 32·5 c.c. of an 18 p.c. solution of sodium chloride contain 5·85 grams of sodium chloride; 32·5 c.c. of a 55·38 p.c. solution of glucose contain 18 grams of glucose; and 32·5 c.c. of an 18·46 p.c. solution of urea contain 6 grams of urea. The addition of these amounts of crystalloids to the 1000 c.c. of blood in the animal would cause that blood to become decinormal blood solutions of the substances, and these decinormal blood solutions are comparable with the decinormal watery solutions that were in a previous paper investigated on the artificial scheme. Of course solutions in blood thus made are not absolutely accurate, but their accuracy is as great as circumstances will allow. For a double reason it was advisable to have the strong solutions as concentrated as possible, 1st, because the amount of water introduced with the crystalloid was neglected in estimating the amount of crystalloid necessary to make decinormal blood solutions, and therefore the less injected the less the error introduced; and 2nd, because I have already shown[1] that variations in the volume of the blood produce very rapidly marked effects upon the specific gravity of the blood and of muscle, and therefore it was desirable to increase the volume of the blood as little as possible by the mere quantity of fluid injected, besides being necessary that the volume of fluid injected should in every case bear a constant ratio to the volume of the blood of the animal. In making the injections, practically, 1 c.c. over and

[1] *Proc. Physiol. Soc.* May 12, 1894. This *Journal*, xvi.

above the amount of solution theoretically necessary was always taken into the syringe to counterbalance any loss by fluid clinging to the sides of the syringe and remaining in the cannula. The solutions and syringe were always left for some hours in a warm chamber at 37° C. in order to inject the fluids approximately at the blood heat, and also to avoid any error due to variations in the temperatures of the fluids injected. The injections were made immediately after a ten-minute observation of the lymph had been concluded and were carried out as rapidly as possible consistent with safety. Speaking generally the duration of the injection was one to two minutes. In the case of sodium chloride, however, the injection had to be carried out more slowly, owing to the profound—frequently fatal—effects of injecting concentrated sodium chloride solution rapidly into the heart; it generally occupied about four minutes. The injections of ·75 p.c. sodium chloride solution and 2·25 p.c. glucose solution were carried out in the same way as those of the strong solutions, excepting that the amount injected was always one-third of the calculated volume of blood of the animal. The injection of such quantities naturally occupied a longer time than injection of the small quantity of concentrated solution.

The injections, when more than one substance was investigated upon the same animal, were not made in any definite order, but in one experiment for example glucose would be injected before urea, in another urea before glucose, and so on, so as to obviate the possibility that the observed effect of injection of one substance really depended upon the substance previously injected or upon some modification in the animal which it produced. As a rule the time that elapsed between two separate injections was about one hour, but the practical point observed was that the rate of lymph flow should have returned to the normal after any injection before a fresh injection was made.

For those experiments in which it was desired to inject finely divided particles of an insoluble substance into the circulation some trouble was experienced in deciding on the substance to be used. Indian ink was thought of, but was discarded owing to the uncertainty of its composition and the probability that some of the component parts of the pigment as usually obtained would have osmotic properties. These by actual experiment it was found could not be removed even by prolonged dialysis, and it is probable that in part they are bound up with the gum used in preparing the pigment. It was however found that if lampblack be mixed intimately with a solution of gum acacia, the paste so formed when diluted freely with water forms an extremely

fine suspension: 2 c.c. of a solution of picked gum acacia in water (about the consistency of olive oil) were diluted with 198 c.c. of distilled water; this mixture has a specific gravity of about 1002 and is the "gum solution" referred to in Figs. 12 and 13 and the accompanying text. Other 2 c.c. of the same solution of gum acacia in water were placed in a mortar and 3 grams of lampblack added; these were kneaded with the pestle till a paste was formed and the whole was washed with 198 c.c. of distilled water into a flask, taking care to clean out the mortar well. On shaking vigorously the whole of the lampblack goes into suspension. After filtering through several layers of filter paper, "gum solution" and lampblack suspension were dialysed for the same length of time against distilled water. The composition of the two fluids was therefore identical, except that in one case solid particles of an insoluble substance in an extremely fine state of division were suspended in the fluid. By injecting first the "gum solution" and observing its effects, and afterwards the lampblack suspension and observing its effects, the true effect of the lampblack particles *per se* might be inferred. Owing to the importance of determining that the lampblack itself possesses no osmotic power special determination of the initial rates of osmosis of the suspending fluid and the suspension were made. They were as follows:

Gum Solution.		*Lampblack suspension.*	
At 0 h. edge of fluid stood at	0 mm.	At 0 h. edge of fluid stood at	0 mm.
After 6 h. ,, ,,	25¾ ,,	After 6 h. ,, ,,	22½ ,,
,, 7 h. ,, ,,	30 ,,	,, 7 h. ,, ,,	26 ,,
,, 24 h. ,, ,,	63½ ,,	,, 24 h. ,, ,,	55 ,,
Average initial rate of osmosis per hour = 2·65 mm.		Average initial rate of osmosis per hour = 2·3 mm.	

Since the same gum solution was used in both cases and the volume in the case of the lampblack suspension was less by the volume of the lampblack present, the rates of initial osmosis should theoretically be approximately identical but the rate should be a little slower with the lampblack suspension. This is seen to be the case, and the relative positions of the edges of the fluids at intermediate periods of the 24 hours show that the final results obtained are in substance correct. The lampblack as such is therefore inert so far as osmosis is concerned and is not contaminated by any crystalloid which exerts an appreciable osmotic power. It follows therefore that any differences obtaining between the results of injecting gum solution and lampblack suspension must occur independently of osmosis, and if no difference occur it

indicates that a mechanical irritant cannot produce a modification in the lymph flow, and the possibility that that increased lymph flow which results from injection of a crystalloid into the circulation may ultimately depend upon the osmosis which can be caused by the crystalloid but cannot be caused by the insoluble lampblack.

In this series of experiments the dogs were all about 7 kilos in weight and 50 c.c. were always used for an injection. The fluids were injected at the body temperature.

Section II. Results of Experiments.

A. The effects of injecting weak equimolecular solutions of sodium chloride and glucose into the circulation, in amounts bearing a constant ratio (1:3) to the computed volume of the blood.

(*a*) *The specific gravity of the arterial blood.* The specific gravity of the arterial blood falls with absolute regularity as the result of injection, but the actual amount of fall differs in different cases. The fall as shown by Sherrington[1] and myself[2] independently is of short

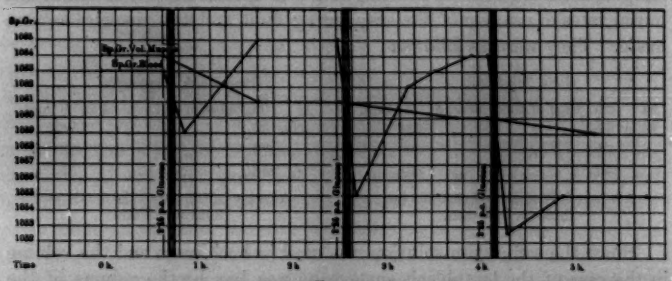


Fig. 3.

Curves showing the specific gravity of blood and of voluntary muscle before and after injection of weak glucose solution. A quantity equal to one-third of the computed volume of the blood was injected during the time represented by each shaded portion[3].

duration, the specific gravity rising again so rapidly that after about half-an-hour the specific gravity of the blood has again reached, or nearly reached, its original height. The curve (Fig. 3) given is a

[1] *Loc. cit.* [2] *Proc. Physiol. Soc., loc. cit.*

[3] The shaded portions of this and the succeeding figures indicate the time and duration of intravascular injection of those substances whose names are placed on the left-hand side of each individual shaded spaces.

representative one, and besides the initial fall shows that after injection the specific gravity of the blood may end by reaching a higher point than before injection. Since v. Brasol[1] has pointed out that glucose leaves the blood very rapidly, so that two minutes after injection a quarter of the amount injected has left the blood, it is extremely improbable that this rise in specific gravity after the injection of weak solutions of sodium chloride or glucose depends upon the presence of the crystalloid in the blood. The cause of this phenomenon is discussed later. This result frequently occurs after repeated injections provided sufficient time (1–1½ hours) be allowed to intervene between separate injections: thus, for example, in an experiment in which two injections of 2·25 p.c. glucose solution were made in 2½ hours the initial specific gravity of the blood before injection was 1059, and the final after the two injections had been given was 1063. The curve given above only shows this effect after the first injection, though the specific gravity of the blood after the second injection ended by reaching a higher point than obtained at the commencement of the experiment. But it shows in a very marked degree another fact, namely, that this result does not occur after an indefinite number of injections. After two or more injections the fall in specific gravity which results (in part at any rate) directly from the introduction of the large quantity of fluid of lower specific gravity into the circulation may only be partially compensated, and the specific gravity of the blood remains for a considerable time far below its original height. Further, the curve illustrates two other points commonly noticed, 1st that the fall in specific gravity of the blood observed within five minutes after the end of injection is less after the earlier than it is after the later injections, and 2nd that the recovery of specific gravity is not so rapid after later injections as it is after earlier ones; thus, after the first injection the recovery of specific gravity in one hour was 6 points, whereas in the same time after the third injection it was only 2½ points.

It appears therefore that large injections of weak equimolecular solutions of sodium chloride and glucose into the circulation cause an initial fall in specific gravity of the arterial blood, which in about one hour is over-compensated for after the first and frequently after the second injection, but after later injections the specific gravity of the blood though it tends to recover itself does so more slowly and

[1] *Du Bois Reymond's Arch.*, 1884. Phys. Abth. p. 211.

less completely. The immediate effect of repeated injections is to cause a greater fall in specific gravity in the later than in the earlier injections.

(*b*) *The specific gravity of voluntary muscle.* The effect of large injections of the solutions into the blood is to cause a fall in the specific gravity of muscle, but this is not invariably the case; on one occasion a small rise was produced, and on four occasions no modification of the specific gravity showed itself as the result of the first injection, though a fall occurred after subsequent injections. The fall, too, is not regular; as in the case of the blood a difference can be seen between the effects of earlier and of later injections. This is clearly seen in Fig. 3, but the difference from the specific gravity of the blood is in the converse sense, for the fall in specific gravity of muscle is greater after the earlier injections, less after the later ones. Muscle differs too from blood in the fact that the tendency towards recovery of specific gravity is not commonly seen during the course of an experiment, though it occasionally happens. It is probable that the reason of this lies in the fact that the period between successive injections is too short. The amount of fall in specific gravity of muscle differs very considerably according to whether the animal has been deprived of water or no, the temperature and humidity of the air, etc. If the air be at a high temperature and far removed from the saturation point and the animal have been deprived of water for 24 hours before the experiment, the fall in specific gravity of muscle is far greater than if the opposite conditions obtain. On the other hand, the outflow of lymph is much less with the former set of conditions, and since this is an essential point in the investigation, as has been said in Section III., water was always given *ad libitum*.

The conclusion therefore is that injections of large quantities of weak solutions of sodium chloride and glucose cause a fall in specific gravity of voluntary muscle, and that fall is greatest with the earlier injections of a series, but with later injections it becomes less and may in some cases be entirely wanting.

(*c*) *The lymph.* As far as concerns the amount of the lymph flow after injections such as those we are now considering, a great difference is observable between the effects of earlier and later injections of a series. Fig. 4, *A* gives a representative curve of the course taken by the lymph flow after earlier injections. It is seen that a rise takes place, which gradually increases until it reaches its maximum in the second period

of ten minutes after the injection. The rise commences during the injection and such curves as the one given are invariably seen. The descent from the maximum is far more gradual than the ascent; the duration of time before the normal is reached varies somewhat, but as a rule it is about one hour; the greater portion of the descent to normal however is complete during the first half-hour after the maximum has been reached. Later injections (Fig. 4, *B*) show a much more rapid ascent, the maximum outflow occurring during the first ten minutes; the actual maximum reached, too, is higher than after early injections. The return to normal is also more sudden, so that the apex of the tracing given by the lymph flow after later injections forms a far acuter angle than the apex of the tracing after earlier injections. With reference to the increase of lymph flow as compared with the amount of lymph formed before injection, it is obviously only fair to compare the effects of the first injection in an experiment, since it is possible that the effects of this may not have completely passed off before a second injection is given, though in the actual experiments care has been taken to avoid this source of error as far as possible. Nevertheless we have seen that the muscle at any rate is lowered in specific gravity as the result of an injection, and the amount of lymph flow after an injection is greatly affected by the amount of water already present in the tissues. The actual numbers obtained as representing the ratio of the amount of lymph formed during the half-hour immediately preceding a first injection of weak sodium chloride solution to the amount of lymph formed during the half-hour immediately following that injection were in six unselected experiments as follows:—1 : 2·5, 1 : 2·3, 1 : 3, 1 : 3, 1 : 2·8, 1 : 2. In other words, injection of weak sodium chloride solution in amounts proportionate to the computed volume of the blood of the animal leads, in different animals, to approximately the same relative increase in the lymph flow.

The effect of large injections of weak equimolecular solutions of sodium chloride and glucose upon the specific gravity of the lymph is invariably an immediate fall. As great a fall as 5 points has been observed. There is occasionally a tendency for the specific gravity of the lymph to rise after the initial fall, but whereas in the case of the specific gravity of the blood this is the rule, in the case of the specific gravity of the lymph it is far more uncommon. Moreover in no case has there shown itself after injection a higher specific gravity of the lymph than before; in other words, even after the first injection the specific gravity of the lymph rarely rises towards its original height,

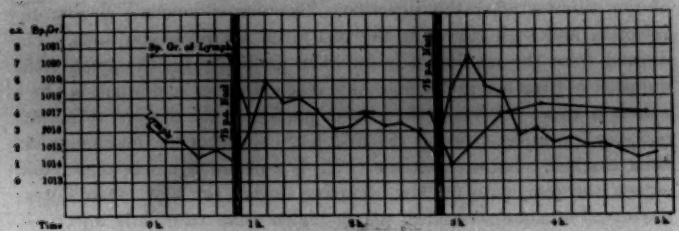


FIG. 4 *A*.

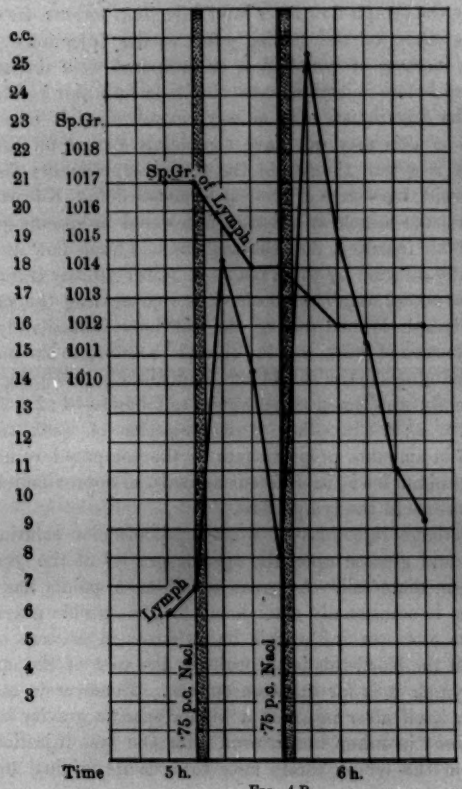


FIG. 4 *B*.

Curves to show the effect of injection of large quantities of weak sodium chloride solution upon the lymph-flow and upon the specific gravity of the lymph. *A*, after earlier injections of a series; *B*, after later injections of the same series.

never reaches it, and much less does it ever exceed it. The curve given by the specific gravity of the lymph over a series of injections is therefore one of a more or less regular descent. Whether the fall after earlier injections in a series is greater than that which occurs after later injections, or *vice versa*, is doubtful, since no definite law in this respect has shown itself.

We may conclude therefore with regard to the effect of large injections of equimolecular solutions of sodium chloride and glucose that they lead to an increase in lymph flow which is smaller, reaches its maximum later, and shows a more gradual return to the normal level after the earlier injections of a series than after later injections; that proportionate injections of the same solutions lead to approximately the same relative increase in the amount of lymph flow after injection to the amount of lymph flow before injection; and that the specific gravity of the lymph as the result of injections gradually falls.

(*d*) *The blood-pressure.* Any permanent effect of large injections such as we are now considering upon the arterial blood-pressure is practically wanting. A slight rise occurs, as is well known, during the actual duration of the injection, but this is immediately readjusted on completing the injection, and with this exception the pressure in the carotid remains constant. So far as concerns the pressure in the inferior vena cava (Fig. 5) there is an initial rise commencing with the injection, continuing throughout it, reaching its maximum as the last drops of the injection are thrown into the vein, and giving place to a fall immediately the injection is stopped. The return to normal is usually complete within ten minutes from the end of injection, and from that time onwards a further fall may take place. This may occur after several successive injections; it was observed on 12 out of 17 experiments in which the venous pressure was taken. The return to normal is not always so rapid as has been stated to be the rule; thus on one occasion it was 1½ hours after injection before the venous pressure again reached the level which obtained before injection. A rule to which only two exceptions have been met with is that earlier injections in a series cause a greater rise in the venous pressure than later injections. This is well shown in the curve given, which is typical. Excepting in one case in which glucose solution was injected I have not found that the length of time before the venous pressure returns to its original level is greater after later than after earlier members of a

series of injections. In this exceptional case the complete return to normal after the first injection took 12 minutes, after the second 1¼ hours, after the third it had not returned to normal when the experiment was concluded, 1 hour and 40 minutes after the injection

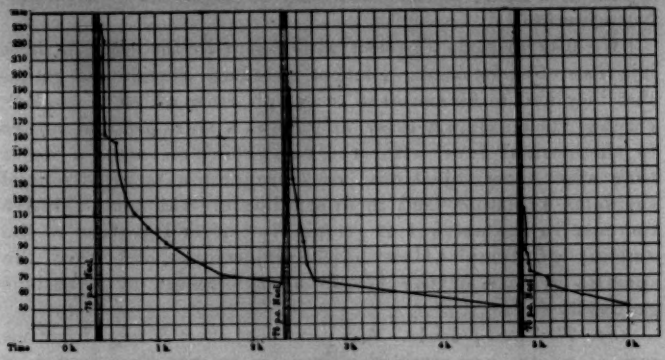


FIG. 5.

Curves showing the effect of large intravascular injections of weak sodium chloride solution upon the pressure in the vena cava inferior.

had been given. The pressure in the portal vein has, for reasons that have already been mentioned, not been investigated in a sufficient number of cases to speak with certainty, but in the four experiments in which it was taken the rise which occurred after injection was somewhat greater and lasted somewhat longer than that which occurred in the case of the inferior vena cava. As far as the return to normal is concerned after large injections of fluid it was complete earlier in my experiments than in those detailed by Bayliss and Starling[1].

SUMMARY.

If we sum up the changes observed as the result of intravascular injections of large quantities of 0·75 p.c. sodium chloride solution and of an equimolecular solution of glucose in amounts proportionate to the computed volume of the animals' blood (1 : 3) they are as follows:— The specific gravity falls in the case of arterial blood, of voluntary muscle and of lymph, but whereas the specific gravity of the blood rises rapidly after the initial fall and frequently reaches a higher level than

[1] This *Journal*, XVI. 159. 1894.

before injection, the specific gravity of the lymph rarely rises and never overshoots the mark, and the specific gravity of muscle almost invariably when it has fallen remains stationary. The lymph flow is increased and the maximum outflow of lymph occurs usually in the second period of ten minutes after earlier injection of a series, usually in the first period of ten minutes after later injections. The carotid blood-pressure is practically unchanged, but the pressure in the vena cava rises rapidly, reaches its maximum with the termination of injection, and returns to normal generally within about ten minutes after injection. After reaching the normal level in the majority of cases it also descends below it, and later injections of a series lead to a smaller rise of pressure than earlier injections. In different animals comparable intravascular injections of ·75 p.c. sodium chloride solution lead to approximately the same relative increase in the amount of the lymph flow.

B. *The effects of injecting concentrated equimolecular solutions of sodium chloride, glucose and urea, into the circulation in amounts sufficient to cause the blood to form in each case an approximately decinormal (blood) solution of the substance.*

(*a*) *The specific gravity of the blood.* In spite of the fact that the specific gravity of the solutions injected was considerably higher than that of the blood (the specific gravity of the glucose solution was 1185) it was only on two out of the thirty-one experiments in which strong solutions were injected—both as the result of injecting 18·46 p.c. solution of urea—that the immediate observed result was a rise in specific gravity of the blood. Five times the specific gravity of the blood neither rose nor fell, two of which were the result of injection of urea solution, three as the result of injection of glucose. In the case of sodium chloride a fall was invariably observed. An analysis of the experiments, therefore, so far as this point is concerned gives, that of 11 experiments with sodium chloride a fall was always observed; of 9 experiments with glucose a fall occurred 6 times, and neither a rise nor fall 3 times; of 11 experiments with urea a fall occurred 7 times, neither rise nor fall, twice, and a rise, twice. This however only applies to the results obtained very rapidly after injection. Later, whether the specific gravity of the blood has fallen, remained stationary or risen there is a rise, and this rise is sufficient in many cases to cause the specific gravity of the blood some time after an injection to stand at a higher level than it did before. These points are

well illustrated in the curve *A* given in Fig. 6. But the blood plasma (as represented by the blood serum) does not quite coincide with the blood as regards the changes that take place in it (Fig. 6, *B*). It is seen

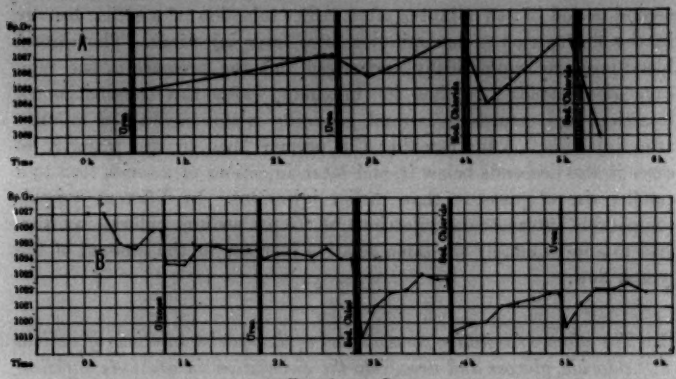


FIG. 6. *A*, *B*.

Curves showing the effect of injections of concentrated equimolecular solutions upon the specific gravity of arterial blood (*A*), and arterial blood-serum (*B*). Equal quantities of each solution were used for each injection in the same animal, and the quantity used for injection in the two animals was always $\frac{1}{110}$th of the body weight. Immediately after injection, therefore, the blood became, theoretically, a decinormal (blood) solution of the substance injected.

that a fall in specific gravity occurs, that it tends to recover itself, but in no case does the final specific gravity reach the specific gravity before injection, so that the *total* effect of the injections is to lower the specific gravity of the blood plasma. The initial fall in specific gravity is however better seen with blood serum than with the blood itself. Coincident with the greater *frequency* with which a fall in specific gravity of the blood is seen after injection of sodium chloride than after injection of glucose, and after injection of glucose than after injection of urea, a comparison of the *amount* of fall in such cases in which a fall occurs in the specific gravity of the blood serum shows that the fall is greatest with sodium chloride injections, less with glucose injections, and least with urea injections. The same holds good with the absolute maximal fall observed throughout the research; with sodium chloride injection it was 6 points, with glucose injection 3 points, with urea injection 2 points. The fall takes place with extreme rapidity; this is exceptionally well shown in curve *B*, where the specimen of blood—

taken from the femoral artery—was obtained in less than a minute after completion of the injection into the external jugular vein. Only sufficient time intervened to enable me to put down the syringe and take up the test-tube in which the blood was collected. In this respect my experiments entirely confirm Leathes[1] in his statement that "the time necessary for this transfer of fluid" (*i.e.* from the tissues to the blood vessels as the result of the injection of glucose) "is inappreciable."

Injections of concentrated, equimolecular glucose, sodium chloride, and urea solutions in strictly proportional amounts are therefore seen to produce different degrees of modification of the specific gravity of the blood and the blood-serum, the effect being most marked with sodium chloride, next with glucose, and least with urea. In all cases the general tendency is to produce at first a fall in specific gravity of the blood and blood-serum, which takes place with extraordinary rapidity, but the later effect is to lead to a rise in specific gravity, which in the case of the blood may lead that fluid to be of higher specific gravity than before the injection was given. A rise is also seen later in the specific gravity of the blood-serum, but it never succeeds in fully compensating for the initial fall.

(*b*) *The specific gravity of voluntary muscle.* Here the effect of injection of concentrated solutions is markedly different from the effect when weak solutions are injected. A rise in specific gravity now occurs

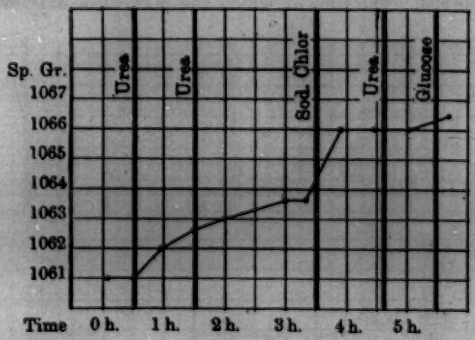


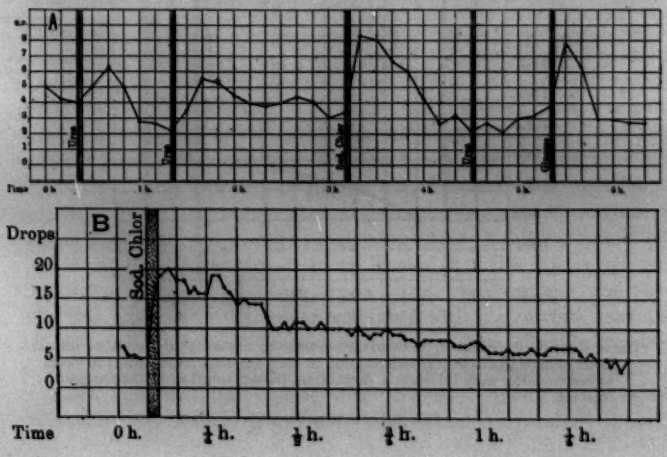
FIG. 7.

Curve showing the effect upon voluntary muscle of injecting concentrated solutions of crystalloids into the circulation in such amounts as to cause the blood to become after each injection a decinormal (blood) solution of the substance injected.

[1] This *Journal*, XIX. 5. 1895.

with almost invariable regularity. Nevertheless differences show themselves according as earlier or later members of a series are examined. Thus in the representative curve given (Fig. 7) the first injection of urea led to a total rise in specific gravity of the sartorius muscle of 1·5 points, the second to a rise of 1 point, the third produced no effect. Further it shows that urea does not produce so much modification of specific gravity as sodium chloride, which in the curve given caused as the result of its injection a rise in specific gravity in the same muscle of 2·5 points. The effect of injecting glucose is not well seen in this curve since it was injected very late in the experiment, but it shows that even when urea will not produce a rise in specific gravity glucose can produce a rise of half a point. This is a matter of extreme importance and attention will be directed to it later. Lastly, no tendency towards a fall is to be seen; throughout the whole of a series of injections the specific gravity of the muscle rises, most rapidly at first, less rapidly afterwards. This is what would be expected, bearing in mind that the ultimate source of the lymph is the tissues and that during an experiment not only is the lymph removed from the animal but also no water is given.

(c) *The lymph.* Fig. 8, *A*, *B*, *C* and *D*. Excepting in three cases after injection of urea, and one after injection of each sodium chloride and glucose in which the amount of lymph obtained from the thoracic duct showed no alteration, an increase in the amount of outflow was invariably observed. The time at which the maximum outflow occurred



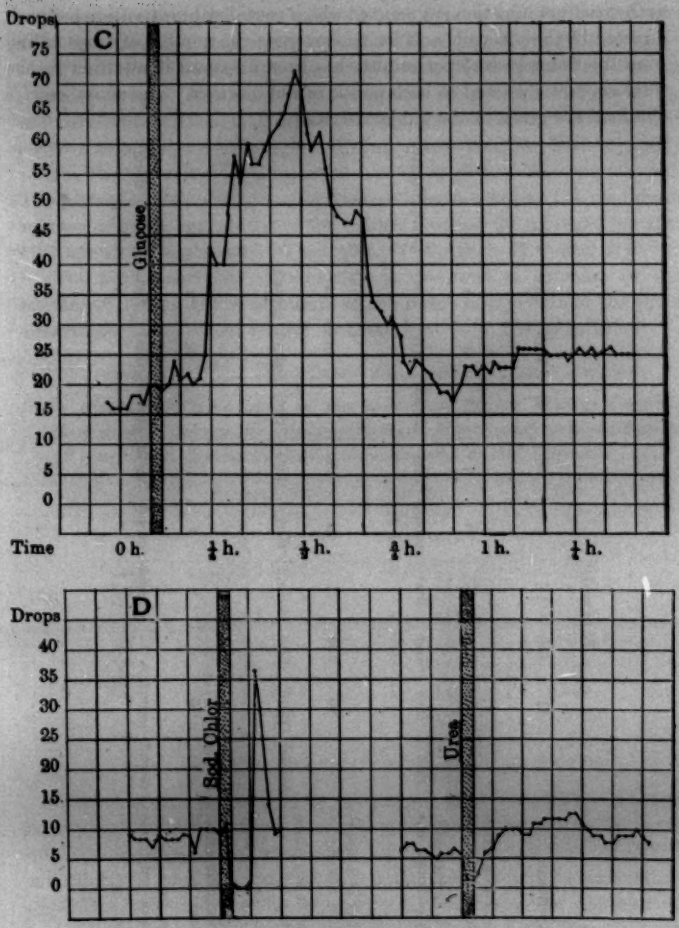


FIG. 8. *A*, *B*, *C*, *D*.

Curves showing the effect of injecting into the circulation equal quantities of equimolecular concentrated solutions of crystalloids upon the lymph flow. (Cf. Fig. 7, which was taken from the same experiment.) For further details see text.

as the result of injection is a point to which considerable attention has been directed in the research, and for this purpose the number of drops falling from the thoracic duct per minute has been observed in addition to the total amount collected in each period of ten minutes. The actual results obtained are given in the subjoined table.

TABLE I.

No. of Experiment	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Sodium Chloride.											
No. of minutes after end of injection before the maximum number of drops appeared	6	5	3	3	14	7	12	3	2	32	31
Period of ten minutes during which maximum quantity of lymph was collected	first	first	first	first	second	first	second	first	first	fourth	fourth
Glucose.											
No. of minutes after end of injection before the maximum number of drops appeared	—	20	? 30	17	22	16	6	20	14		
Period of ten minutes during which maximum quantity of lymph was collected	second	third	fifth	second	third	third	first	third	second		
Urea.											
No. of minutes after end of injection before the maximum number of drops appeared	9	16	1	9	7	10	14	20	3	28	28
Period of ten minutes during which maximum quantity of lymph was collected	second	second	?	second	second	fourth	second	third	first	third	third

It appears from the above table that no general law can be deduced as to the time that elapses between the end of injection and the maximum outflow of lymph, since the range is too wide to be fairly thrown into an average. One can however say that the general tendency is for the maximum outflow of lymph after an injection to occur earlier when sodium chloride has been injected than when urea has been injected; earlier when urea has been injected than when glucose has been injected. The averages of the lengths of time after injection before the maximum number of drops appeared give for sodium chloride 9·8 minutes, for urea 13·2 minutes, for glucose 16·4 minutes. (Cf. Fig. 8, *B*, *C*, and *D*.)

The next point for consideration is the relative increase in the amount of lymph flow that occurs as the result of injection of the three substances. The actual results obtained were as in the table below.

TABLE II.

Table showing the proportion of the amount of lymph flowing from the thoracic duct during the half-hour previous to an injection to the amount of lymph flow during the half-hour subsequent to that injection.

No. of Exp.	Sodium Chloride	Glucose	Urea
I	1 : 2·49	1 : 1·18	1 : 1·24*
II	1 : 1·17	1 : 4·15*	1 : 1·91
III	1 : 2·21	1 : 0·62	1 : 0·97
IV	1 : 0·43	1 : 2·22	1 : 5·20*
V	1 : 2·7	1 : 2·41*	1 : 4·95
VI	1 : 1·93	1 : 1·74	1 : 1·45*
VII	1 : 2·1	1 : 1·1*	1 : 0·9
VIII	1 : 1·57	1 : 1·14	1 : 0·9
IX	1 : 1·95	1 : 1·8*	1 : 1·82
X	1 : 2·7		1 : 1·3
XI	1 : 1·86		1 : 1·05

The most obvious point in these figures is their variability. Though, no doubt, the general effect of injection of any of the substances in the quantity specified is approximately to double the amount of lymph collected, yet marked cases (glucose 2, and urea 4 and 5) occur where the effect produced was far greater, while examples are not wanting (sodium chloride 4, glucose 3, urea 3, 7, and 8) in which after the injection of the particular substance an actual domination in lymph production was observed. For strict comparison, however, only first injections in a series should be taken. These are only available in sufficient numbers in the cases of glucose and urea, since the possibility of death occurring

as the result of the introduction into the heart of the concentrated sodium chloride solution rendered me unwilling after all the preparations had been made for the experiment to run the risk of obtaining only one result, and that of possibly a doubtful value. An asterisk is affixed to the cases of first injections of glucose and urea. It would appear that urea leads to a somewhat greater increase of lymph flow than glucose. In spite however of the obvious disadvantages incurred thereby, it seems more satisfactory to compare the effects of the three substances upon one and the same animal. Such means of comparison are given in the following groups.

TABLE III.

Table showing the proportion borne by the amount of lymph flow from the thoracic duct during the half-hour previous to injection into the same animal of equal quantities of equimolecular solutions of sodium chloride, glucose, and urea, to the amount of lymph flow subsequent to such injections.

	Sodium Chloride	Glucose	Urea
Animal I.	1 : 2·1	1 : 2·2	1 : 1·24
Animal II.	1 : 1·57	1 : 1·1	1 : 0·9
do.	1 : 1·95	1 : 1·4	1 : 1·82
Animal III.	1 : 2·7	1 : 1·8	1 : 1·3
do.	1 : 1·86	—	1 : 1·05
Average	1 : 2·03	1 : 1·6	1 : 1·26

From these figures it is clearly seen that the large whole figure in urea IV (Table II) has vitiated the whole conclusion, and that in the same animal and using equal amounts of equimolecular solutions, sodium chloride leads to a greater relative increase of lymph flow than glucose, and glucose than urea. Nevertheless it must be borne in mind that such injections as we are now considering in a certain number of cases lead to an actual diminution in the amount of lymph flow.

A point however of extreme interest in connection with the lymph flow and its possible relationship to osmosis concerns the behaviour of the lymph flow during and immediately after injection. If osmosis occur as the result of an injection into the circulation, for a short—possibly a very short—time during or after injection an absorption of water from the lymph spaces should take place. This fluid not passing off in the usual way by the lymphatics and thoracic duct should lead to a temporary diminution in the amount of lymph escaping by the thoracic duct. This point has not escaped the notice of Heidenhain, and with

reference to it he says, "as soon as any change in the length of time between individual drops of lymph was observed it consisted only in a shortening[1]." In my experiments the number of drops per minute has been taken with great care, and though generally speaking my observation agrees with that of Heidenhain, in a certain number of cases this was not so[2]. As a rule from the very beginning of the injection the number of drops falling from the thoracic duct increases, sometimes gradually, sometimes by leaps and bounds. The following gives the results obtained: out of 30 injections, in 21 at no time after commencing the injection or during the first 5 minutes after completing the injection did the number of drops per minute fall below the level obtaining before the injection. In 9 cases, on the other hand, the number of drops per minute, either during injection or during the first 5 minutes subsequent to its completion, fell below the level obtaining before injection. Two of these occurred after injection of glucose, three after injection of urea, four after injection of sodium chloride. Of the cases in which a rise occurs this is not invariably unbroken, as in Fig. 8, *B* (though in the majority of cases it is so), but as shown in Fig. 8, *C*, there is a broken ascent. Fig. 8, *D*, gives examples in which a diminution in the outflow from the thoracic duct took place.

The specific gravity of the lymph undergoes changes which are well

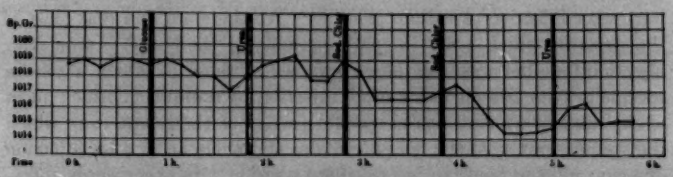


FIG. 9.

Curve showing the variations in specific gravity of the lymph as the result of injection of equal quantities of equimolecular solutions of sodium chloride, glucose and urea in strengths sufficient to cause the blood to form after each injection a decinormal (blood) solution of the substance injected.

seen in the representative curve given (Fig. 9). During the first period of ten minutes after the injection had been made into the circulation

[1] *Loc. cit.* p. 261.

[2] Cohnstein (*Pflüger's Arch.* LIX. 519, and LX. 295) finds a marked slowing of the lymph stream during and immediately after injection of salt solution into the circulation. He does not say whether he finds this invariably to be the case. Heidenhain (*Verhdlg. d. internat. Med. Cong.* Bd. II. Abth. 2, p. 57) completely denies the occurrence of a slowing.

the specific gravity of the lymph falls only in a minority of cases. Out of 27 experiments in which the specific gravity of the lymph was taken a rise occurred in 15, the specific gravity remained stationary in 7 and fell in 5; of the 7 cases in which the specific gravity remained stationary, 4 were after injections of urea, 2 after injections of sodium chloride, 1 after injection of glucose; of the 5 cases in which a fall occurred, 3 were after injections of sodium chloride, 2 after injections of glucose. As regards the substances individually, of 9 examinations of the specific gravity of the lymph after injection of sodium chloride a rise was observed in 4 cases, a fall in 3, and the specific gravity remained stationary in 2; after injection of glucose the specific gravity of the lymph out of 9 occasions rose on 6, fell on 2, remained stationary on 1; and after injection of urea out of 9 occasions it rose on 5 and remained stationary on 4. Nevertheless on one occasion with each of the three substances the "stationary" specific gravity gave place to a rise in specific gravity during the second ten minutes, and twice with urea a stationary specific gravity gave place to a rise in specific gravity still later. An initial fall in specific gravity was followed by an over-compensating rise on one occasion (glucose). This leaves only 4 occasions upon which a distinct fall was observed as the result of injection, and 2 occasions on which a constant specific gravity was maintained throughout. Of the four cases in which a distinct fall occurred in the specific gravity of the lymph 3 were after injection of sodium chloride. We therefore find that most of the cases in which a fall in specific gravity of the lymph is seen after injection together with the fewest number of those in which a rise in specific gravity is seen, occur after injection of sodium chloride; most of the cases in which a rise in specific gravity of the lymph or in which the specific gravity of the lymph remains stationary occur after injection of urea; glucose holds an intermediate position. In other words, intravascular injection of urea has a greater tendency to raise the specific gravity of the lymph than injection of an equal quantity of an equimolecular solution of glucose, and glucose a greater tendency to raise the specific gravity of the lymph than sodium chloride.

The actual maximum rise or fall as the result of injection was about 3·5 points in specific gravity. In the curve given the variations are not so marked as those frequently seen, but the general shape of the curve is representative of the greater number of experiments, and shows that there is as a rule a rise in specific gravity which may reach its maximum during the first ten minutes, or in certain cases, *e.g.* urea, at a later time.

(*d*) *The initial rates of osmosis of blood-serum and of lymph.* These were investigated by the method already described, and the curves given (Fig. 10) show the complete set of investigations carried out upon the same animal. The curves are representative ones, and in interpreting them it must be remembered that the samples of blood were taken at the times indicated on the curves but that the lymph was collected during the ten minutes preceding and ending at the times indicated on the curves.

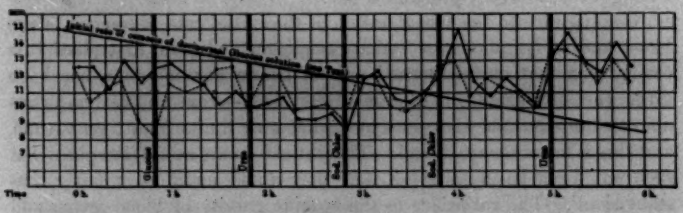


FIG. 10.

Curves showing the variations in the initial rate of osmosis of blood-serum and of lymph in an animal in which equal amounts of equimolecular solutions of sodium chloride, glucose and urea were injected intravenously. With reference to the level of decinormal glucose solution cf. p. 424.

Confining our attention first to the initial rate of osmosis of the blood-serum it is seen that it is considerably modified by the injections as a whole; whereas before the injections in the curve given it was less than the initial rate of osmosis of decinormal glucose solution, after about three injections it rose above the level of the initial rate of osmosis of glucose, and remained above it, gradually rising higher and higher until the termination of the experiment. This is what might be expected considering that the water removed in various ways from the animal (lymph urine, respiration) was not replaced during the experiment, and that crystalloids in large quantities had from time to time been added to the blood, but an examination of the specific gravity of the blood-serum (Fig. 6, *B*, was taken from the same experiment) shows that the rise in initial rates of osmosis of the blood-serum is accompanied by a diminution in the specific gravity of the blood-serum. The increased initial rate of osmosis cannot therefore be due to an increased proportion of crystalloid in the serum, since under those conditions the specific gravity of the blood-serum would be raised. The experiments detailed in a former paper give data[1] whence it is reasonable to conclude that this

[1] Lazarus-Barlow. This *Journal*, *loc. cit.*

combination of circumstances must be due to a diminution in the proteid composition of the blood-serum. Since albumen impedes osmosis, as has been shown, and also has a higher specific gravity than water, a diminution of albumen in the solution alone is sufficient to explain both the diminution in specific gravity and the higher initial rate of osmosis of the blood-serum after a series of injections, but since no actual estimations of the amount of proteid leaving the blood vessels in the lymph have been made this explanation must only be regarded in the light of a suggestion, however probable a one.

Coming now to the consideration of the initial rate of osmosis of the blood and of lymph under various conditions. In the first place it is clearly seen that in the normal animal before any injection has been given, the initial rates of osmosis of the blood-serum and of lymph are not constants but vary between somewhat wide limits from time to time, and this I have always found. Theoretically one would imagine that such would not be the case but that they would maintain a fairly constant level[1]. The variations in the specific gravity of blood-serum and lymph (especially the former) however show that there is additional reason beside actual investigation for regarding the above statement as accurate. Since the volume of lymph obtained from the thoracic duct rises and falls as the initial rate of osmosis of the blood-serum rises and falls, it is probable that the initial rate of osmosis of the blood-serum depends upon the actual removal of water in the lymph.

After an injection the initial rate of osmosis of the blood is modified, but contrary to what one would expect the modification is not invariably in the direction of a rise. Thus it will be noticed in the curve given that on two occasions (once each with urea and sodium chloride) the initial rate of osmosis diminished. There can be no doubt that this is due to the extreme rapidity with which water enters the blood vessels after an injection; could the blood be obtained before any water has entered the circulation it is certain that the blood-serum would be found in all cases to have undergone a rise so far as initial rate of osmosis is concerned. But since the mere dilution of the blood-plasma by a fluid containing proportionately more water and less proteid (*i.e.* the lymph outside the blood vessels) lowers the proportion of albumen in the blood-plasma, this very dilution should militate against a diminution in the initial rate of osmosis of the plasma (or serum). The diminution of initial rate of osmosis of the blood-serum seen in certain cases after

[1] If the lymph were thrown into the blood, as in the normal animal, it is highly probable that such variations would either be absent or at all events less marked.

intravascular injection of a crystalloid therefore becomes the more incomprehensible.

There is not only however a general tendency (in spite of the exceptions) for the initial rate of osmosis of the blood-serum to increase immediately after injection of a crystalloid into the circulation, there is a rule to which no exceptions have been found for the initial rate of osmosis of the blood-serum to increase further at a later period of the injection. This frequently occurs during the first period of ten minutes after the injection, so that it has invariably happened that the initial rate of osmosis of the serum of blood taken 11 minutes after the termination of injection is higher than that taken 1 minute after injection. Occasionally there is a further increase observed in the initial rate of osmosis of the blood-serum, but this is not so common. No comparisons can be made between the amounts of modification in the initial rate of osmosis of the blood-serum after injection of sodium chloride, glucose and urea. The curve given by the initial rate of osmosis of the lymph corresponds very closely with that given by the blood-serum, so that a detailed description is not necessary. Thus, the initial rate of osmosis of the lymph during an experiment in which several injections of crystalloids are made into the circulation rises gradually throughout and without exception during the ten minutes after each injection. But the point of greatest interest and importance lies in the fact that the initial rate of osmosis of the lymph collected during any period of ten minutes is not indicated by the initial rate of osmosis of blood-serum obtained during the first minute of *that* period, but by the initial rate of osmosis of the blood-serum obtained during the first minute of the *next* period. From this it follows that variations in the initial rate of osmosis of the blood-serum are preceded by variations in the initial rate of osmosis of the lymph and not *vice versâ*, and therefore that the lymph regulates the initial rate of osmosis of the blood.

This is a matter so opposed to our present conceptions of the relations between blood and lymph that it is necessary to examine the matter more closely. In the curves given, out of 35 occasions on which the initial rate of osmosis of the blood-serum and of lymph were taken, on only 7, viz. at 0 h. 20′, 0 h. 50′, 1 h. 20′, 1 h. 30′, 2 h. 10′, 4 h. 20′, 4h. 30′, did the initial rate of osmosis of the lymph collected during the preceding ten minutes on each occasion travel in an opposite direction to the initial rate of osmosis of the blood-serum obtained one minute *after* the corresponding period of ten minutes. In all the other cases

the directions taken by the two curves are identical. Now a closely similar proportion obtained in all other experiments made, so that of the total number of occasions (57) in which the initial rate of osmosis of blood-serum and lymph were estimated 75 per cent. showed that the change which the initial rate of osmosis of the blood-serum at any given time would take was indicated by the changes that occurred in the initial rate of osmosis of the lymph collected during the *preceding* ten minutes. This being the case it becomes very doubtful whether the modifications in the initial rate of osmosis of the blood-serum registered immediately after the injection of any crystalloid into the circulation depend upon that injection at all. In the case of the injection of urea at 5 h. (Fig. 10) the increased initial rate of osmosis of the blood-serum from 10 to 13·5 might naturally be regarded as due to the presence of the additional crystalloid in the blood, but seeing that a similar rise occurred in the initial rate of osmosis of the lymph before any urea was introduced into the circulation, and bearing in mind the large amount of evidence which has just been shown to exist for the view that the initial rate of osmosis of the lymph preindicates the initial rate of osmosis of the blood-serum, it becomes conceivable that the actual rise seen in the case of the blood-serum ought correctly to be correlated with the rise in the initial rate of osmosis of the lymph, and be entirely dissociated from the fact that urea has been injected into the blood. That there are difficulties in the way of such a view is only too obvious, but the indications given by the curves seem too clear to be lightly thrown over. Under these circumstances the initial rate of osmosis of the blood-serum would be so rapidly modified by any changes that occurred in the lymph that it would really be difficult to ascribe any of the observed changes in the initial rate of osmosis of the blood-serum directly to the injection.

With regard to the lymph the matter is simpler, for, as has already been shown, the initial rate of osmosis of the lymph invariably increases, and is at its maximum during the first ten minutes after injection; it is therefore easier to suppose that it bears a definite relationship to the injection preceding it. Moreover, as has been seen, the specific gravity of the lymph rises at the same time.

In the last place the initial rate of osmosis of the lymph may be greater than that of the blood. On 22 occasions (3 being before any injection was given at all) out of the 57 in which observations were made the initial rate of osmosis of the lymph was greater than the initial rate of osmosis of the blood-serum obtained at the end of the

period, and on 29 occasions (3 of which were before any injection was given) greater than the initial rate of osmosis of the blood-serum obtained at the beginning of the period. Whether therefore the view be taken that the composition of the lymph is regulated by the blood, or the composition of the blood is regulated by the lymph, the fact remains that the initial rate of osmosis of the lymph is frequently greater than that of the blood. But since in the normal animal the lymph is introduced into the vascular system at the termination of the thoracic duct such differences cannot long remain unadjusted. The very fact of the termination of the thoracic duct in the vascular system gives a further indication that the composition of the lymph has a large share in regulating the composition of the blood.

The conclusions to which one is therefore led are, 1st, in the normal animal but with the thoracic duct opening outwards the initial rates of osmosis of the blood and lymph vary from time to time within rather wide limits; 2nd, the initial rate of osmosis of the lymph may be higher than that of the blood-serum; 3rd, as the result of a series of injections of crystalloids in an animal the initial rates of osmosis of blood-serum and of lymph increase gradually; 4th, any change in the initial rate of osmosis of the blood-serum is preceded by a similar change in the initial rate of osmosis of the lymph.

(*e*) *The blood-pressure.* So far as concerns the arterial blood-pressure the result of injecting strong solutions of sodium chloride, glucose and urea such as those we are now considering, is the same in all cases and consists in a maintenance of the blood-pressure unchanged during the actual period of injection, followed—immediately after or at the latter end of the injection—by a rapid and considerable fall in blood-pressure, which lasts for about two minutes, and then recovers itself. After the recovery the arterial pressure may rise for some short time a little above the level obtaining before injection, but such a termination I have found to be unusual; as a rule the recovery is complete but not excessive, and the normal blood-pressure is fully attained within 5 minutes from the end of injection.

The fall of blood-pressure is greater after injection of sodium chloride than after injection of either glucose or urea.

The pressure in the inferior vena cava rises as the result of injection of each of the substances in concentrated solution, and to a more marked degree than after injection of weak solutions. Maximum

pressures of 300 mm. of magnesium sulphate and upwards were frequently observed, especially after injection of sodium chloride. The return to normal was as rapidly completed as in the greater number of experiments where weak solutions were injected, but varied in

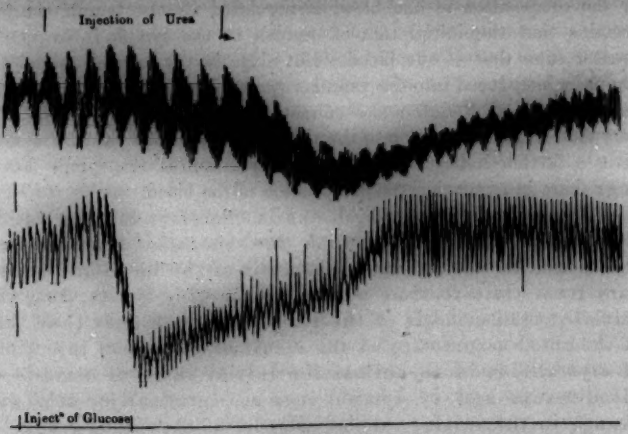


FIG. 11.

Curves showing the effects of injecting concentrated solutions of urea and of glucose into the circulation upon the blood-pressure taken in the carotid artery.

different cases; nevertheless it was generally effected in about 10 minutes. In one experiment however it was not complete till 40 minutes after the solution (concentrated glucose) had been injected, but a second and exactly similar injection of glucose given 1 h. 40′ after the first injection led to a rise in the venous pressure which was above the level obtaining before injection during only five minutes. The general shape of the curve is similar to that shown in Fig. 5.

It may therefore be stated that injection of concentrated solutions of sodium chloride, glucose and urea at the end of or immediately after injection lead to a fall in blood-pressure which is soon readjusted, and that no further changes in arterial blood-pressure occur; and to a marked rise of venous pressure, which is usually readjusted completely in about ten minutes, and which differs solely from the effect of injecting large volumes of weak solutions in the greater height to which the venous pressure rises when smaller volumes of concentrated solutions are injected.

C. The effect of injecting fine insoluble particles in a state of suspension into the circulation.

These injections were made with the object of determining, if possible, whether an increase of lymph flow can be brought about by mere mechanical irritation of the vascular endothelium. Lampblack was chosen as the solid insoluble substance for injection, not only because of its fine state of division but also of its amorphism. It was undesirable to use any material which might lacerate the endothelial walls. The method of preparation of the suspension has already been given (pp. 428, 429). The experiment is not an ideal one, by reason of the rapidity with which the solid particles are seized upon by the leucocytes and removed from the circulation. No lampblack has ever shown itself in the lymph, and it vanishes from the blood in a remarkably short time. My observations on these points have been confirmed verbally by Dr L. E. Shore, who with another object in view injected Indian ink into the circulation some years ago. As a matter of fact on removing portions of the organs from the body as soon as 20 minutes after a single injection of lampblack suspension and examining microscopically, the leucocytes (*e.g.* in the spleen) are seen to be crammed with black particles, whereas only here and there a trace is visible in the blood-filled vessels. One would therefore assume that such differences, if any, as may occur between the effects of injecting a quantity of the suspending-fluid (gum-solution) and the effects of injecting an equal quantity of the lampblack suspension under exactly similar conditions should show themselves principally during the early period after an injection.

In Figs. 12 and 13 are given the effects of such injections upon the specific gravity of the blood, of voluntary muscle and of lymph, and upon the lymph flow, considered both in reference to the amount of lymph flowing from the thoracic duct during periods of ten minutes and to the number of drops of lymph falling per minute from the thoracic duct.

So far as the specific gravities of the blood and voluntary muscle are concerned there is no need for detailed description of the curve, since the curves of these two correspond almost exactly with the curves obtained after injection of weak solutions of crystalloids (Fig. 3). One important difference however is to be observed. No initial fall in specific gravity of the blood is to be observed after the injection of the

solutions, which is gradually compensated for by a rise. The blood curve is therefore here simpler than in Fig. 3, but the essential points that a rise above the normal occurs after the earlier injections and a gradual fall occurs after the later injections are very noticeable. The

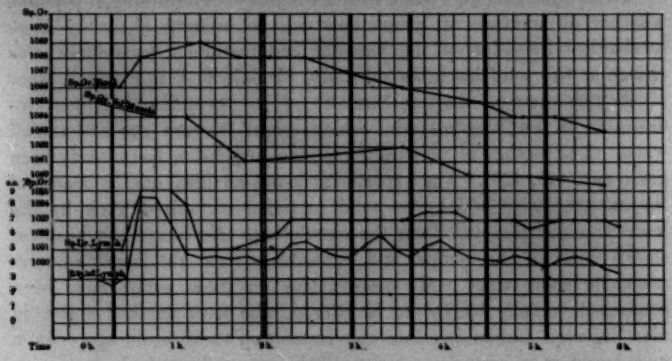


FIG. 12.

Curves showing the effect of injecting gum solution and suspension of lampblack in that gum solution into the circulation, upon the specific gravity of blood, of muscle and of lymph, and upon the amount of lymph flow per 10 minutes from the thoracic duct. The first, third and fifth shaded spaces correspond to injections of 50 c.c. of gum solution; the second, fourth and sixth to injections of 50 c.c. of lampblack suspension.

small rise in specific gravity of muscle which is shown after the 2nd and 3rd injections I am inclined to regard as due to error of observation, but the curves have been given in their entirety as they were obtained from the particular experiment and are especially of value as giving absolutely comparable results. The fall in specific gravity of the voluntary muscle as the total result of all the injections is well shown. No difference is to be seen between the effects of injecting gum-solution and injecting lampblack suspension. With regard to the curve given by the specific gravity of the lymph it follows in a remarkable manner the curve given by the quantity of lymph flow from the thoracic duct, even to the small changes visible after the 4th, 5th and 6th injections. In spite of the low specific gravity of the fluids injected (gum solution 1002, lampblack suspension 1004) more lymph is poured out of the thoracic duct, and that lymph contains proportionally more solids than in the normal lymph. The total effect of all the injections upon the specific gravity of the lymph is to raise it somewhat.

Eliminating the effect of the first injection, which is probably due at all events in part to the fact that the economy is unused to the presence of gum solution, the amount of lymph flow per ten minutes like the specific gravity of the lymph of muscle and of blood shows no difference whether gum solution or lampblack suspension be injected into the circulation. In each case the amount of flow is slightly increased, and the increase diminishes with each injection, until after the 6th injection the variation is no more than occurs in a normal untouched animal. Nor is there any difference in the later stages after an injection between the rates of lymph flow as measured by the number of drops per minute falling from the thoracic duct (Fig. 13). The curves thus given—with the exceptions to be mentioned—are so similar that it has been thought unnecessary to reproduce them in full. The most marked difference however obtains between the immediate effects of injecting gum solution and injecting lampblack solution. The results obtained in the experiment which is represented by Fig. 12 are given in Fig. 13. It is seen that there is a marked diminution in the outflow from the thoracic duct during or immediately after the injection of lamp-

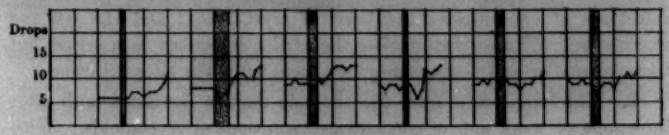


FIG. 13.

Curves showing the immediate effects of injecting gum solution and a suspension of lampblack in the same gum solution upon the rate of lymph flow as estimated by the number of drops falling per minute from the thoracic duct. The first, third and fifth shaded spaces correspond to injections of 50 c.c. of gum solution; the second, fourth and sixth to injections of 50 c.c. of lampblack suspension.

black suspension, whereas such a diminution is either wanting or is but doubtfully marked after injection of gum solution. This seems to imply that after injection of lampblack suspension fluid leaves the lymph spaces and enters the blood vessels; but since as has been shown on p. 429 the osmotic equivalent of the lampblack suspension is slightly less than the osmotic equivalent of the gum solution with which that suspension is made up, this diminution of lymph flow occurring during or immediately after injection into the circulation of the lampblack suspension cannot depend upon osmosis. The importance of this fact and the clearness with which the curves speak are considerable.

Section III. Conclusions.

We are now in a position to consider how far an answer can be given by the experiments to the question with which we set out, viz. What parts are played by osmosis and filtration in lymph-formation? It will be of assistance to collect here the most important of the conclusions to which the experiments have led; they are as follows:—

1. As the result of injecting into the circulation large quantities of weak solutions and small quantities of concentrated solutions of crystalloids, and weak solutions of gum, the specific gravity of arterial blood falls with great rapidity, but later recovers itself, and after the first, and frequently the second, injection ends by being higher than it was before injection. With concentrated injections of equal amounts the initial fall in specific gravity of the blood is greater after injecting sodium chloride, less after injecting glucose, least after injecting urea.

2. The specific gravity of voluntary muscle falls after large injections of weak solutions, and weak solutions of gum, rises after small injections of concentrated solutions of crystalloids; and the fall and rise respectively are greatest after the earlier injections in a series, but become smaller and perhaps may be absent after the later injections.

3. The lymph flow from the thoracic duct is increased both after injections of weak and of concentrated solutions of crystalloids. With weak solutions the maximum outflow of lymph occurs during the second period of ten minutes after the injection is ended in the case of earlier members of a series, during the first period of ten minutes in the case of later members of a series. The absolute maximum outflow of lymph is greater in the case of later members of a series than in the case of earlier members. With concentrated solutions the absolute maximum outflow of lymph is less in the case of later members of a series where the same substance is injected than in the case of earlier members. The maximum increase of lymph occurs at varying times after the injection, but the maximum number of drops falling per minute from the thoracic duct occurs with sodium chloride on an average ten minutes after injection, with urea thirteen minutes after injection, with glucose sixteen minutes after injection.

4. In the majority of cases the curve given by the number of drops falling per minute from the thoracic duct after injection of concentrated solutions of sodium chloride, glucose and urea forms an ascent (which

may be broken or unbroken) towards the maximum. In a certain number of cases injection is accompanied or followed by an initial diminution in the outflow from the thoracic duct.

5. Sodium chloride leads to a greater relative increase of lymph flow than glucose, and glucose than urea, after intravenous injection.

6. Comparable injections of a weak solution of sodium chloride in different animals lead to approximately the same relative increase in lymph flow.

7. When injection of a concentrated solution of urea leads to no rise in specific gravity of muscle and no increase in lymph flow, a rise in specific gravity of muscle and an increase of lymph flow may be brought about by injection of an equal quantity of an equimolecular solution of glucose. Examples of the converse have also been found. (The second injection was not made until the effects of the first had fully passed off.)

8. The initial rate of osmosis of the lymph collected from the thoracic duct may be higher than the initial rate of osmosis of the blood-serum.

9. The serum of blood taken immediately after injection of a concentrated solution of a crystalloid into the circulation may have a lower initial rate of osmosis than it had before such injection.

10. Variations in the initial rate of osmosis of the lymph precede variations in the initial rate of osmosis of the blood-serum.

11. Neither concentrated nor weak solutions of the substances when injected into the circulation in the quantities given in the body of the paper lead to a rise in arterial blood-pressure. Concentrated solutions lead to a temporary fall in blood-pressure, which is re-adjusted in about five minutes from the end of injection. Injection of both kinds of solution leads to increase of pressure in the vena cava inferior which is often very marked, is at its maximum at the termination of injection, begins to fall immediately injection is stopped, has returned to its normal level in about ten minutes, and the fall is often continued so that the venous pressure remains for a considerable time after injection at a lower level than obtained before injection.

12. An insoluble substance such as lampblack when injected into the circulation does not modify the lymph flow except during or immediately after injection, when there occurs an initial diminution in the number of drops of lymph falling per minute from the thoracic duct.

Confining our attention in the first place to the positive evidence for the occurrence of osmosis and filtration in the animal body as the effective agents of lymph-formation, we see that undoubtedly the most powerful arguments for their occurrence are found in the facts that the falls in specific gravity of the blood after injection of sodium chloride, glucose and urea respectively, correspond in their relative magnitudes with the initial rates of osmosis of those three substances and that the ratio of the amount of lymph formed after injection to the amount formed before injection of any of the three substances, is greater as the initial rate of osmosis is greater, less as it is less. In other words, the amount of fluid entering the blood vessels after injection of any substance varies directly as the initial rate of osmosis of the substance injected; the amount of fluid passing through the blood vessels by filtration also varies directly as the rise of pressure produced by the osmotic flow, *i.e.* as the initial rate of osmosis of the substance injected[1]. It is necessary to point out however that if the falls in specific gravity of the blood occurring after injection of the crystalloids be dependent upon the physical process of osmosis the membrane composing the vessel wall must be more nearly akin to a copper ferrocyanide membrane than to prepared peritoneal membrane of calf. The order of magnitude in initial fall in specific gravity of the blood is the same as the order of magnitude of initial rates of osmosis with sodium chloride, glucose and urea with a copper ferrocyanide membrane, but when peritoneal membrane of calf is used on the osmometer it has been clearly shown in a previous paper that the relation of the initial rates of osmosis of glucose and sodium chloride is different. Whereas with a copper ferrocyanide membrane the initial rate of osmosis with sodium chloride is greater than the initial rate of osmosis with glucose, when peritoneal membrane of calf is used the converse is the case. Urea maintains the same final position with both kinds of membrane. So far therefore as these two sets of data are concerned one is justified in concluding that the increased lymph flow which occurs as the result of injection of a crystalloid into the circulation depends upon increased osmosis and increased filtration

[1] The falls in specific gravity of the blood do not correspond with the *final osmotic pressures* of the solutions, for then they should be approximately identical after injection of glucose and urea: this is not found to be the case, but on the contrary the fall in specific gravity of the blood observed after injection of urea is markedly less than that observed after injection of glucose. This point however does not vitiate the argument, since it has already been repeatedly urged that questions of final osmotic pressure can hardly come in, in biological problems—and it is certainly impossible to suppose that they play any important part in such experiments as those which have been detailed.

through a vessel wall which in nature is akin to a copper-ferrocyanide membrane. One has all the more reason for coming to this conclusion because the falls in specific gravity of the blood occur with such extreme rapidity that little time has been given for removal of the crystalloid from the blood, and therefore the process at work is as far as possible simplified.

Besides the two facts above given, positive evidence of considerable importance is found in the fact that injection of quantities of weak sodium chloride solution which bear a constant ratio to the volume of the blood, in different animals, leads to approximately the same relative increase of lymph flow from the thoracic duct. From this fact it might fairly be reasoned that by the injection the intravascular pressure is raised to the same extent in each animal, and that the correspondence of the ratio between the increase in lymph flow after injection and the amount of lymph flow before injection in all the animals shows that the same increase of intravascular pressure leads to the same rate of filtration through the vessel wall.

Positive evidence, but of an inferior kind, is also yielded by those cases in which an initial diminution of lymph flow occurs after injection of the crystalloid into the circulation. In view of the facts that such cases form a minority of all the cases observed and that a similar initial diminution in the lymph flow occurs after injection of such a substance as insoluble lampblack this kind of evidence loses a good deal of its weight.

But apart from the inherent improbability that the blood vessel wall with its stomata is more closely akin to a chemical membrane such as copper ferrocyanide than to an animal membrane such as prepared peritoneal membrane of calf there is a large number of facts which seem to array themselves against such a purely physical explanation of lymph-formation.

In the first place, evidence has already been given in a former paper[1] which bears directly upon the question we are now considering. Thus it was found (p. 790) that when a ligature is placed round the hind limb of a dog sufficiently tight to raise the venous pressure to a height of 50 mm. of mercury (the arterial pressure being 100 mm.) the amount of lymph flow from the lymphatics of the limb taken on the distal side of the ligature "is either identical with or slightly less than the amount secreted when the venous pressure is normal: in no case was it greater."

[1] Lazarus-Barlow. *Phil. Trans. Roy. Soc., loc. cit.*

Again, it was found that "the immediate effect (*i.e.* the direct effect independent of any other interference with the animal) of section of the sciatic nerve is a sudden fall in the amount of lymph flow" (p. 801). Now under either of these conditions there is reason to believe that the pressure in the capillaries of the limb is increased, and yet this increase in the intracapillary pressure is unaccompanied by any increase in the amount of lymph flow. But the question of exudation was not only considered from the side of the lymphatics, it was also considered from the sides of the tissues and blood. These also showed no modification, and the conclusion was inevitable that increase in pressure in the capillaries of a limb for at any rate one hour does not lead to increased exudation from those capillaries.

In the second place, after an intravascular injection, whether it be of 1 p.c. "gum solution," or of ·75 p.c. sodium chloride solution or 2·25 p.c. glucose solution and in amount equal to one-third of the theoretical volume of the blood, or be of 18 p.c. sodium chloride, 55·38 p.c. glucose or 18·46 urea solution and in amount equal to one four-hundredth of the body weight (one thirty-first of the theoretical volume of the blood) there is the undoubted fact that the initial fall in specific gravity of the blood which except in the case of "gum solution" occurs immediately after the injection, is frequently over-compensated for, and the specific gravity of the blood an hour or an hour and a half after the injection is higher than it was before the injection. That this final superiority of specific gravity depends upon an actual diminution of the volume of the plasma is shown by two experiments, in which blood taken from the femoral artery before and after injection of normal saline solution into the external jugular vein was centrifugalised under exactly similar circumstances. The plasma before the injection constituted in the two experiments ·74 and ·71 of the whole blood, while after injection in each respective experiment the plasma only constituted ·71 and ·65 of the whole blood. The difficulty of finding a satisfactory physical explanation of this fact is very great.

The next point for consideration is the relative increase in the amount of lymph flow. Though this bears a certain relationship to the amount of initial fall in the specific gravity of the blood which occurs after injection of either glucose sodium chloride or urea, and in a series of averages varies directly as the initial rate of osmosis of the substance injected, the very considerable differences obtaining not only after several injections into the same animal but to a more marked extent after approximately comparable injections of the same substance into different

animals, are so great that it becomes doubtful whether an average is of any real value. But further, when we find for example that injections of urea, in other respects comparable, in one experiment lead to a five-fold increase in the amount of lymph, and in three others lead to an actual diminution in the amount of lymph, it becomes doubtful whether one is justified in merging the two kinds of result in an average. The cases, five in number, in which an actual diminution in the amount of lymph flow occurred after injection are extremely important; they show that some markedly different process is at work than is ordinarily the case. They cannot be explained by the assumption that no more water is present in the tissues for the purposes of osmosis, since injection of an equal quantity of an equimolecular solution of another chemically "indifferent" substance may lead to a marked effect[1]. This is very clearly shown in Fig. 8, *A*, where an injection of urea led to an actual diminution in the amount of lymph flow, while 40 minutes later, when the normally increased outflow of lymph due to injection of urea should have long passed its maximum, and when also as the result of loss by lymph, respiration etc. the available water in the animal was, of course, less, an injection of glucose equimolecular with the urea solution and introduced into the circulation in exactly the same amount led to more than double the amount of lymph flow. If it be objected that the initial rate of osmosis of glucose is greater than the initial rate of osmosis of urea, examples can be given where injection of urea caused a greater increase of lymph flow than a previous injection of glucose. Thus in one experiment injection of glucose led to an increase of lymph flow which compared with the lymph flow before injection was 1·14 : 1, whereas an injection of urea given 2 hours later caused an increase of lymph flow which compared to the lymph flow before injection was 1·82 : 1. Such facts as these cannot easily be explained upon purely osmotic or filtration principles. It is evident too that the vascular system does not react so readily to later injections of the same substance as to earlier injections. This has already been noted by Heidenhain with reference to other crystalloids[2]. It obviously calls to our mind the fatiguing of a muscle under repeated stimuli of constant strength. This phenomenon however may be seen in purely physical membranes such as prepared peritoneal membrane of calf, as has elsewhere been noted, and under other purely

[1] In view of the wrong conception involved in the use of the term "isotonic" as to the relative amounts of osmosis caused by such equimolecular solutions of indifferent substances at pressures occurring within the animal body, I hesitate to apply to them the physical term "isotonic," which is justifiable only so far as *final* osmotic pressures are concerned.

[2] *Loc. cit.*

physical conditions (*e.g.* "fatiguing" of Crookes' vacuum tubes) and therefore it is necessary to use such an analogy with caution. In the opposite direction, viz. an exaltation of the amount of increase in lymph flow after a second injection of the same substance, examples are not wanting. Thus in one animal the first injection of urea caused a slight diminution of the amount of lymph obtained from the thoracic duct, while a second injection given an hour later, when by previous experience in addition to the actual data furnished by the specific gravity of the blood etc., it was known that the effects of an earlier injection had passed off, led to very nearly double the amount of lymph flow.

But obstacles to the view that osmosis is accountable for the increased volume of water in the blood immediately after injection of a concentrated solution of a crystalloid into the circulation come from a consideration of the curves given by the number of drops that fall per minute from the thoracic duct. Granting the support to the mechanical view by the definite cases in which a diminution in the lymph flow is one of the earliest observed facts after the injection has been made there is still a large majority of cases in which such a diminution is completely wanting and in which a more or less steady and continuous increase in the number of drops falling from the thoracic duct is observed from the very first. Further, the time that elapses between the end of injection and the appearance of the maximum number of lymph drops per minute from the end of the thoracic duct constitutes a difficulty. One might reasonably imagine that this length of time should theoretically vary inversely as the rate of osmosis of the substance injected. Now an average of all the cases in which sodium chloride was injected certainly shows that the maximum number of drops appeared earlier after injection of this substance than after injection of either glucose or urea, but contrary to what should occur under purely physical circumstances, corresponding averages after injections of glucose and urea show that the maximum number of drops per minute makes its appearance earlier with urea than with glucose. The questionable value of such averages has however already been pointed out.

Lastly, we come to consider the evidence given by observations on the blood-pressure, and at the outset I may say that my experiments on this point have led me to a conclusion diametrically opposed to that which Starling has deduced from his experiments. As Starling and Bayliss have shown, venous pressures give a much more probable indication of capillary pressures than do arterial pressures. Nevertheless the presence of muscular elements in the walls of the veins and the

definite evidence of the existence of vaso-motor control of the portal veins adduced by Mall[1] must make us careful in drawing conclusions concerning capillary pressures even from observations on venous pressures. So far however it is the most reliable evidence at our disposal. Now an examination of the curves given by the pressure in the vena cava and the lymph flow after injection of a concentrated solution of a crystalloid shows that in the vast majority of cases the venous pressure has returned to the normal long before the maximum outflow of lymph occurs.

That the actual pressure in the capillary system is at its greatest when the pressure in the vena cava is highest, is not shown by this latter fact alone, for an active contraction of the vascular system might take place, but when it is found that the maximum intravenous pressure and the maximum volume of the blood, as shown by the specific gravity of the blood, coincide in point of time, it is reasonable to assume that if filtration occur through the vessel wall that this will be the time at which the maximum amount of filtration will take place. That in this respect my experiments agree so far as observations are concerned with those of Starling is shown by a comparison of my curves with the curve given by him[2]. Though my results differ from his in that I find the maximum output of lymph occurring later and the return to normal of the pressure in the vena cava occurring earlier than he does, yet from his own curve it is clearly seen that the maximum outflow of lymph occurred five minutes after the pressures in the vena cava and portal vein had reached their maxima and had begun to fall. But further my curves agree with the one given by Starling, in that they all show that the amount of lymph flow may be greatly increased long after the venous pressure has returned to normal. Thus in Starling's curve the pressure in the vena cava had returned to normal 29 minutes after the termination of injection, but the lymph flow was still at this time at least four times as great as it was previous to injection; and even 54 minutes after the termination of the injection and 25 minutes after the pressure in the vena cava had returned to normal the lymph flow was more than double the amount previous to injection.

The points to which I have just alluded seem to me to be of extreme importance. It is not sufficient to show that after an injection of glucose or any other substance a rise of venous pressure *and* an increased outpouring of lymph occur, and thence to conclude that the latter depends upon the former as the result of filtration through the vessel

[1] *Du Bois Reymond's Arch., Phys. Abth., Suppl.*, 1890, p. 57.

[2] This *Journal*, XVII. 1894, Plate I, Fig. I.

walls; before such a conclusion is justifiable it must be shown that the known laws of filtration are fulfilled throughout. The patent facts that the time of maximum intravascular pressure is not coincident with the maximum outpouring of lymph and that the lymph flow remains increased long after the pressure in the systemic veins has returned to normal, I regard (in spite of the small rise of pressure which according to Starling's curve obtains in the portal area after the pressure in the vena cava has returned to normal) as strong arguments that the two factors, capillary pressure and lymph-formation are *not* inter-dependent.

To sum up. In favour of the occurrence of osmosis and increased filtration as the effective factors in causing the increased outflow of lymph that is seen after injection of a crystalloid into the blood we have,

1. The evidence given by the lowering in specific gravity of the blood after injection of comparable quantities of equimolecular solutions of sodium chloride glucose and urea, which is found to vary directly as the initial rate of osmosis (through a copper ferrocyanide membrane) of the substance injected (p. 438).

2. Those cases in which injection of a crystalloid is followed by a temporary diminution in the outflow of lymph from the thoracic duct (p. 445).

3. The fact that the increase in amount of lymph flow after injection of sodium chloride, glucose and urea, in a series of averages varies directly as the amount of the lowering of the specific gravity of the blood and therefore as the initial rate of osmosis (through a copper ferrocyanide membrane) of the substance injected (p. 444).

4. The fact that with large injections of weak sodium chloride solution in amounts bearing a constant ratio to the theoretical volume of the blood of the animal, in different animals the ratio between the amount of lymph formed after injection to the amount of lymph formed before injection is practically a constant (p. 433).

Against the view that osmosis and increased filtration alone account for the increased amount of lymph flow after intravascular injection of a crystalloid we have,

1. The fact that the initial fall in specific gravity of the blood which follows immediately upon injection of a concentrated solution of sodium chloride, glucose or urea, is frequently over-compensated, and that this over-compensation occurs after large injections of fluids having a considerably smaller initial rate of osmosis than either blood plasma or lymph (pp. 431, 437, 453).

2. The cases (forming a large majority) in which injection of a concentrated solution of sodium chloride, glucose or urea is not followed by an initial diminution in the outflow of lymph from the thoracic duct, but by a constant and immediate increase of outflow (p. 445).

3. The fact that injection of a crystalloid may in a minority of cases actually lead to a diminution in the total amount of lymph flow from the thoracic duct, and that after such a diminution of lymph flow injection of an exactly comparable quantity of another crystalloid given when the effect of the first injection may be presumed to have passed off, may lead to a large outpouring of lymph. (p. 461.)

4. The fact that in the same animal when the amount of water present in the tissues and available for osmosis has been diminished by loss of lymph &c., injection of a crystalloid with a lower initial rate of osmosis may lead to a greater increase in the amount of lymph flow than that to which injection of an equal amount of another crystalloid with a higher initial rate of osmosis has led when the amount of water present in the tissues and available for osmosis had not been diminished by loss of lymph &c. (p. 461.)

5. The fact that the initial rate of osmosis of the lymph may be higher than the initial rate of osmosis of the blood serum. (p. 450.)

6. The fact that the maximum outflow of lymph does not occur at the time of maximum intravenous pressure, and that the outflow of lymph remains increased long after the intravenous pressure has sunk to normal. (p. 463 and elsewhere.)

7. The fact that considerable increase in venous pressure in the hind limb of a dog continued for an hour, and that section of the sciatic nerve led in neither case to an increase of lymph flow from the lymphatics of that limb.

8. The fact that an initial diminution in the outflow of lymph which might in the case of injection of a crystalloid into the circulation be regarded as evidence of the occurrence of osmosis is seen after injection of an insoluble substance such as lampblack. (p. 455.)

9. The fact that the points in which the process may easily be explained by osmosis can only be so explained upon the inherently improbable assumption that the blood vessel wall is more nearly akin to a chemical membrane such as copper ferrocyanide than to an animal membrane such as prepared peritoneal membrane of calf.

(The expenses of this research were defrayed by a grant from the British Medical Association.)

NOTES ON RENNET AND ON THE COAGULATION OF MILK. By ARTHUR EDMUNDS, B.Sc. (Lond.)

(*From the Physiological Laboratory, King's College, London.*)

I. *Rennin in the Testis.*

In his *Text-book of Chemical Physiology*, p. 580, Prof. Halliburton states that pieces of testis possess the power of curdling milk. This statement has been recently contradicted by Dr R. Peters[1] who writes "A testis removed from a dog immediately after death was divided into small pieces and extracted for 24 hours with glycerine; the extract was filtered, but after several hours at 40·5° C. no coagulating action on milk could be observed. Further the fluid squeezed out from a fresh bull's testis when added to milk after neutralisation, produced a similar negative result." From this he concludes that the testis does not contain a milk curdling ferment.

Prof. Halliburton tells me, that the statement was inserted in his book on the authority of a traveller in Italy, who informed him, that the peasants in certain districts were in the habit of using pieces of testis, instead of rennet, in cheese making. Recognising however the necessity of putting the matter on a surer basis, he suggested to me that I should investigate the subject. The two experiments of Peters are inconclusive, because he used fresh tissues; for the extraction of ferments, especially when they occur as zymogens, it is well known that some means such as treatment with dilute acid is necessary, to convert the zymogen into the ferment.

The following is an account of the experiments I have performed. The first testis that I used was from a ram. It had been kept on ice for four days and was without any trace of putrefaction. Portions of this were dried in the sun, fragments of it (and of epididymis), added to milk did not cause clotting. But I obtained a clot by adding to the milk a few drops of an extract of the dried testis made with saturated

[1] *Unters. ü. das Lab, und die labähnlichen Fermente: Preisschrift.* Rostock, 1894.

salt solution. The clot however, took twenty-four hours to form, but was perfectly typical; when thrown into a vessel of water it remained coherent. In many of the experiments subsequently to be described, but not in all, the clots took quite as long to form; it was necessary therefore by control experiments to guard against the error of mistaking for a true clot, the precipitation of the caseinogen caused by the milk turning sour.

Part of the same ram's testis was cut up and extracted with glycerine; the extract alone added to milk caused either no curdling at all or only after twenty-four hours, but the addition of a few drops of a two per cent. solution of calcium or barium chloride as well as the extract, produced a clot within a couple of hours; in control experiments it was found that the same quantity of the calcium or barium salt alone, produced no clotting.

The next experiments I carried out were with rats' testes. The fresh testis added to milk, or the glycerine extract of fresh testis gave negative results; but a glycerine extract of rat's testis which had been treated previously for twenty-four hours with 0·1 per cent. hydrochloric acid gave typical clots, though often only after twenty-four hours had elapsed even if calcium chloride was added as well; the same was true for the 0·1 per cent. hydrochloric acid extract.

Other specimens of the same milk were curdled in a few minutes by commercial specimens of rennet or glycerine extract of stomach. These experiments I take to mean that the testis sometimes contains a very minute trace of rennin or a rennin-like ferment, sufficient however to cause curdling in the presence of excess of calcium.

II. *Rennin in other Organs.*

The preceding experiments naturally led me to investigate whether this property is confined to the testis, and consequently I examined a number of other organs. In each case the organ was divided into small pieces, then treated for twenty-four hours with 0·1 per cent. hydrochloric acid, and then with glycerine. Both the glycerine and the acid extracts were examined, the acid extracts being first neutralised; and in many cases I have succeeded in obtaining extracts active in curdling milk, though as already stated the action was usually very slow. The acid extracts and the glycerine extracts generally gave the same results. In the first series of experiments, extracts were prepared from the liver, lung, muscle, kidney, spleen and ovary, all of which coagulated milk;

but in these experiments the curious and unexpected result was obtained that while the extracts which were used without any calcium salt almost all caused coagulation within fifteen hours, most of those specimens which contained calcium chloride had undergone no change. The extract of muscle however seems, like the testis extracts, to have been markedly helped by the calcium, a good clot forming in less than two hours. It is somewhat remarkable that the calcium chloride should in the case of the testis and muscle accelerate the coagulation, but in that of the other organs retard it.

The dog from which the above-named organs were obtained had been killed in an experiment on intravascular coagulation, but this could not be the cause of the curious result just referred to. I have since then made extracts of various organs from normal mammals (dogs and cats) and in all cases the extracts of numerous organs caused milk to coagulate; and the action of calcium chloride was most erratic; in some cases it appeared to make no difference in coagulation time, in others it accelerated and in others still it retarded the clotting. It is just possible that this is in part due to the differences in amount of the ferment present, the phenomenon having a parallel in the varied effects produced by nucleo-proteids on blood, according to the amount injected.

I also made an experiment with sheep's thymus. This was cut up and extracted with 0·1 per cent. hydrochloric acid. A few drops of this extract together with 0·5 c.c. of a 10 per cent. solution of calcium chloride caused a well-marked curd to form in about ninety minutes. This is the shortest time in which I have been able to obtain a clot from an extract of any organ other than the stomach.

I will give the details of only one other experiment. A cat was killed by chloroform; ligatures were placed around the left half of the liver, and the left renal artery. A stream of salt solution followed by one of tap-water was then injected through the aorta so as to thoroughly wash out the blood from those organs not protected by the ligatures. Extracts were then made from various organs in the usual way, and portions of the extracts added to specimens of milk in test-tubes. In each test-tube were 10 c.c. of milk, and to some 0·5 c.c. of a 10 per cent. solution of calcium chloride was added as well as the tissue extract. The result of the experiment is given in the table. It will be seen that those extracts which were used with calcium chloride, caused on the whole more marked clotting than those which were used without the calcium salt; and that those extracts from tissues freed from blood, were on the whole more active than those from tissues in which the blood had been

allowed to remain. It will also be noted that the extract of blood clot was the least active of any, a fact which I noticed in all my experiments.

Organs	HCl Extracts neutralised		Glycerine Extracts			
	Examined after 24 hours		Examined after 24 hours		Examined after 48 hours	
	No $CaCl_2$	with $CaCl_2$	No $NaCl_2$	with $CaCl_2$	No $CaCl_2$	with $CaCl_2$
Liver, with blood vessels washed out	weak clot	firm clot	contracted clot	contracted clot	contracted clot	contracted clot
Liver, vessels ligatured	nil	clot	nil	nil	contracted clot	contracted clot
Kidney, with vessels washed out	clot	clot	weak clot	nil	contracted clot	firm clot
Kidney, vessels ligatured	firm clot	clot	nil	nil	contracted clot	contracted clot
Brain	firm clot	firm clot	no clot	weak clot	contracted clot	contracted clot
Lymph Glands [mesenteric]	firm clot	clot	weak clot	nil	contracted clot	firm clot
Thyroid	clot	clot	weak clot	nil	contracted clot	firm clot
Small Intestine	weak clot	clot	nil	nil	contracted clot	firm clot
Blood Clot	weak clot	weak clot	weak clot	nil	contracted clot	firm clot

These results are the reverse of what would be expected on the theory that the rennin, like pepsin, had been re-absorbed from the alimentary canal. It may be, however, that rennin though it reaches the tissues *via* the blood and lymph is more or less "fixed" by them, in much the same way as pepsin is absorbed by fibrin from fluids like urine which contain only minute traces of it[1]. The fact that Holovotscheiner and Helwes[2] have obtained from urine a small quantity of a rennin-like ferment appears to me in favour of the re-absorption theory. Why the tissues containing blood yielded less active extracts than those freed from blood, I can only answer by suggesting that the alkalinity of the blood partly neutralised the acid employed and so retarded the extraction, or that glycerine dissolves out from blood other substances besides rennin which inhibit the action of that ferment.

[1] Leo. *Centralblatt med. Wiss.* 1875. 933.

[2] *Pflüger's Archiv*, XLIII. 384. 1888.

The only other alternative to the re-absorption theory as far as I can see is that rennin, or a substance which acts in the same way on milk, is formed in minute quantities in the metabolism of many tissues. In favour of this view it must be admitted that rennin is undoubtedly formed in some situations where it cannot possibly serve the purpose of curdling milk, *e.g.* in certain plants, Ficus etc. Here it must either be formed for some hitherto unknown use, or merely be an accidental product of metabolism. The presence of a milk-curdling ferment in the pancreatic juice can also be of little service to the economy because the milk upon which it would act has already been acted upon by the gastric juice.

In connection with this point it is interesting to note that none of the ferments of the animal body, with the possible exception of trypsin, are absolutely confined to the organ of which they are a characteristic product. Brücke for example has observed the presence of pepsin in muscle and most other tissues, also in the urine. Holovotscheiner[1] has reported a diastatic ferment resembling ptyalin from the urine, while a sugar inverting ferment has been detected by Robertson[2] in a large number of organs, including the whole of the alimentary canal, even the œsophagus, the liver, pancreas, spleen, thyroid, salivary glands, muscle and kidney. To these, as I have pointed out above, rennin must be added as an occasional constituent of practically all tissues and organs.

III. *Solubility of casein.*

In a paper on the Proteids of Milk which appeared some years ago, Prof. Halliburton[3] makes the following statement, "The similarity of caseinogen and casein to myosinogen and myosin appears to me to be exceedingly close." Prof. Halliburton allows me to state what were the experiments that led him to that conclusion.

Caseinogen was prepared by a modification of Hammarsten's method which is described in full in the paper just quoted[4], and dissolved in lime water. On adding rennet to this solution no coagulation occurs; on adding rennet and a few drops of dilute phosphoric acid (to form calcium phosphate), a clot of casein is produced. On taking this clot, washing it free from calcium salts and once more

[1] *Chem. Centralb.* 1886. 327.
[2] *Edinburgh Med. Journ.* Sept. 1894.
[3] This *Journal*, XI. 464. 1890.
[4] This *Journal*, XI. 449. 1890.

dissolving it in lime water, the addition of rennet causes no coagulation; but the addition of rennet *plus* phosphoric acid caused the formation of a curdy precipitate. This may be again dissolved, and the process repeated indefinitely. From this it appeared that re-coagulation of casein could occur in a similar way to that in which the re-coagulation of myosin may be made to take place. Subsequently and after a paper by Dr Ringer[1] on the subject, Dr Halliburton made a further experiment which modified his previous conclusion. He took the solution of casein, and added phosphoric acid alone without any rennet; the calcium phosphate so formed caused an immediate precipitation of the casein. The apparent re-coagulation of the casein by rennet and a calcium salt was really only the precipitation of the casein by the calcium salt; other calcium salts like the chloride will do equally well. Dr Ringer showed that one of the most striking differences between caseinogen and casein is that the solutions of the former are not, but of the latter are readily precipitated by soluble salts of calcium. Hence it is, by such methods, absolutely impossible to investigate whether rennin is capable of re-coagulating casein, because the ferment solution demands the presence of a calcium salt which of itself causes a precipitate. Peters[2] has also taken up this question, and while he adopts Halliburton's nomenclature of caseinogen and casein, he nevertheless maintains that caseinogen cannot be distinguished from casein (as Hammarsten originally stated) by the action of rennin, for solutions of both are, he states, curdled by that ferment. He took the curd produced in milk by the addition of rennet, washed it and dissolved it in lime water. On adding rennet to this solution a heavy precipitate (Fällung) took place. This was again dissolved in lime water, and again gave a precipitate; he repeated this process four times more.

He further states that the preparation of rennet he employed (Witte's Labessenz) gave a clot when added to a solution of caseinogen prepared by Hammarsten's method, without any addition of phosphoric acid, a result never obtained with preparations of rennet used in this laboratory. It therefore seemed advisable to examine Witte's preparation to see if it contained some soluble salt of calcium. So far however I have been unable to obtain any of this preparation.

The most recent paper dealing with the question is one by Dr D. Harris[3]. Though he admits the many and important differences,

[1] This *Journal*, XI. 450. 1890. [2] *loc. cit.*

[3] *Journ. of Anat. and Physiol.* XXIX. 195. 1895.

of solubility etc., between caseinogen and casein, he like Peters, though by a different series of experiments, contends that casein resembles myosin in its capability of being re-dissolved and re-coagulated. He finds that when casein is pounded with water, a creamy fluid is obtained which, when put into the water bath with rennet, sets into a solid; this solid can in its turn be treated in the same way and with the same result, and this can be repeated many times.

I disagree with the deduction drawn from this experiment, on the basis of the following experiments.

(i) A clot of casein was pounded with distilled water, and the creamy fluid thus obtained was divided into two parts. To one part rennet was added, to the other nothing. Both were put into the water bath at 40° C. and in a few minutes both were solid. Harris seems to have performed this experiment also, but he has apparently missed the point of it. In fact pounding a curd with water does not dissolve the casein at all; it merely breaks it up and hastens the separation of the whey; if the mixture is kept quiet the particles simply stick together again, whether rennet is added or not.

(ii) Casein was pounded up as before and the resulting creamy fluid centrifugalised. The fluid which separated out contained only a trace of proteid matter (lactalbumin and whey proteid from the whey) and no clot formed on the addition of rennet.

(iii) Ringer's method for preparing caseinogen is as follows:—The precipitate produced by adding acetic acid to milk is washed with water and ground up in a mortar with calcium carbonate. The mixture is then thrown into excess of distilled water; the fat rises to the surface; the chalk falls to the bottom and the intermediate fluid contains the caseinogen in solution. This coagulates on the addition of rennet and calcium chloride. Dr Ringer[1] states that casein similarly treated with calcium carbonate is almost insoluble in distilled water. This statement I have confirmed. Instead however of pouring the mixture of casein and chalk into a tall vessel of distilled water, I added a small quantity of water and centrifugalised. The fluid obtained was slightly opalescent, owing to some fat which had not risen, but it was practically proteid free and gave no clot on adding rennet.

(iv) The following experiment I have performed at the suggestion of Mr Colls. It consisted in pounding a clot of casein not with distilled water but with a few crystals of ammonium oxalate. The clot

[1] *loc. cit.* p. 477.

broke down into a fluid of the consistency of ordinary milk which does not show the slightest tendency to set once more into a coherent clot. I then filtered the fluid thus obtained and found that the fat was almost entirely retained on the filter while the filtrate was a slightly opalescent liquid which gave strong proteid reactions. It was also readily precipitated by a trace of a calcium salt and by acetic acid. I took another specimen of the dissolved casein clot and centrifugalised it, and here again found that the fat separated from the rest of the fluid. The whole divided into three layers; the fat floated at the surface; a sediment, probably calcium oxalate, sank to the bottom; there was an opalescent liquid between the two.

Microscopical investigation revealed the total disappearance of the casein granules visible in a preparation of a small piece of crushed clot, and also the fact that the fat globules had slightly run together, although on the whole most of them were as free as in normal milk.

This peculiarity of separation into these three layers may be also observed in milk to which rennet has been added after the addition of sufficient ammonium oxalate to prevent coagulation. After standing for twenty-four hours practically all the fat will have risen, leaving the casein in solution as a slightly opalescent liquid.

These experiments I take to mean, that casein is soluble in a solution of ammonium oxalate and that it is merely rendered soluble, probably by the removal of calcium salts, and not reconverted into caseinogen, since it is precipitable by calcium chloride—some of this precipitate would of course be calcium oxalate, but as only a small quantity of ammonium oxalate was originally used, this would only account for a small fraction of the precipitate. They show furthermore that casein is inferior to caseinogen as an emulsifier.

Thus calcium salts can be removed from casein either mechanically, as for example by prolonged washing, or chemically, by the addition of ammonium oxalate, the effect being the same in both cases, viz. the casein is rendered soluble.

IV. *The Relation of Alkali Albumin to Caseinogen.*

The exact relation of these two proteids has given rise to a good deal of discussion. One of the chief reasons for holding that they are different bodies is, that while caseinogen can be curdled by rennin in the presence of a small quantity of calcium salt, alkali albumin is incapable of being so acted upon.

Dr Peters, however (*op. cit.*), states that this distinction does not exist. He prepared alkali albumin (Lieberkühn's jelly) from white of egg; and after a certain amount of washing, dissolved it in boiling water, filtered, precipitated the alkali albumin with acetic acid, and re-dissolved in lime water. This he found gave a flocculent precipitate (Fällung) with neutral rennet, a firm clot with rennet to which a trace of acetic acid had been added, and was unaffected by boiled rennet. We may pass by the fact that Lieberkühn's jelly if thoroughly washed from alkali becomes opaque and tough, somewhat resembling fibrin as obtained from whipped blood, and is not soluble in boiling water; and we take the solution of alkali albumin as prepared by Dr Peters. This solution is in fact precipitated by most of the commercial rennet extracts, but it is also precipitated by the extracts when boiled. In this point it will be noticed I do not agree with Dr Peters. I find further that these extracts contain calcium salts. And it has been shown by Dr Ringer[1] that alkali albumin is precipitable by a trace of a calcium salt, and that the more the reaction of the solution approaches to neutrality, the less calcium is required to precipitate it.

The results obtained by Dr Peters are then, I think, to be explained by the presence of calcium salt in the rennet preparation used by him.

V. *The effect of Peptone on Milk Coagulation.*

If a solution of commercial "peptone" such as Witte's or Grübler's be injected into the circulation and the animal killed by bleeding it is found that the blood has lost the power of clotting and this property can be restored to the blood by the addition of a calcium salt provided that a small dose only of peptone has been injected.

At Prof. Halliburton's suggestion I have undertaken a series of experiments to determine whether "peptone"[2] has any effect on the coagulation of milk by rennin at all comparable to that which it has in the case of blood. The method adopted was to dissolve a known weight of peptone in a known volume of milk and then to dilute this with normal milk to the desired extent, thus obviating the error which would have been introduced by diluting the milk if peptone dissolved in water had been used.

[1] *Proc. Physiol. Soc.* 1891. This *Journal*, XII.

[2] Where peptone is mentioned it should be understood to be in all cases "commercial peptone," which consists mainly of albumoses containing little peptone in the strict sense of the word.

In all cases I found that the action of rennet was retarded by the presence of peptone and those specimens of milk which contained a relatively large percentage of peptone clotted after those specimens which contained a smaller percentage. I give the details of one experiment which illustrates this point. Each tube contained 10 c.c. of

Temperature of bath 40° C.

Number of grams of "Peptone" per 100 c.c. of milk	5	2·5	1·25	·625	0
Exp. commenced at 5.13 p.m.					
5.23 „					clotting commenced
5.27 „					clotting finished
5.33 „				clotting commenced	
5.37 „			clotting commenced		
5.39 „				clotting finished	
5.41 „			clotting finished		
5.57 „		clotting commenced			
5.63 „		clotting finished			
7.10 „	Still fluid. It was however clotted by next morning at 11 a.m.				

milk, a varying quantity of Witte's peptone, and 150 cubic millimetres of rennet (Martindale's).

The next point that naturally occurred was to determine whether the coagulability of milk which has been destroyed by peptone can be restored by calcium in the same way as the action of a small dose of peptone can be neutralised in the case of blood. I find that 0·5 c.c. of a 10 per cent. solution of calcium chloride in 10 c.c. of milk to which the usual quantity, 150 cm. of rennet, had been added will cause rapid clotting even in the presence of 2·5 grms. of peptone per 100 c.c.; with a larger percentage, 5 grms. per 100 c.c., the action of the calcium is slower and more unsatisfactory. At present I can offer no explanation of this peculiar action. I think, however, that the theory that peptone removes or throws out of action the calcium salts which

has been advanced in the case of blood is equally untenable here. I have pounded a clot of casein with peptone, and, although as stated above, ammonium oxalate readily removes the calcium and causes liquefaction, peptone does not cause the slightest alteration in the consistency of the clot.

VI. *Synthesised Colloids and Milk.*

The remarkable colloid substances prepared by Prof. Grimaux, which resemble proteids in so many of their reactions, having been found by Dr Pickering[1] to possess the power of causing intravascular clotting, it was desirable to ascertain if they had any effect on milk. I have experimented with a 0·5 per cent. solution of Grimaux's "colloïde aspartique" prepared by passing a current of gaseous ammonia heated to 170° C. over solid aspartic anhydride, but have only obtained negative results, the milk remaining unaltered at the end of 18 hours.

Conclusions.

(i) A small quantity of milk-curdling ferment can be obtained from other tissues and organs besides the stomach, viz. Testis, Liver, Lung, Muscle, Kidney, Spleen, Thymus, Thyroid, Brain, Blood, Small Intestine, Ovary.

(ii) There is no evidence that casein can be converted into caseinogen and re-coagulated by rennin, the apparent re-coagulation obtained by Peters being probably due to calcium salts present in the rennet extract.

(iii) The statement made by Peters as to the coagulability of alkali albumin by rennin is inaccurate, the coagula he obtained being probably due to the presence of calcium salts in the rennet essence.

(iv) Peptone has a marked retarding effect on coagulation which may be partly, at least, neutralised by the addition of calcium chloride.

(v) Casein is soluble in ammonium oxalate without being re-converted into caseinogen.

(vi) Grimaux's colloïde aspartique has no action on milk coagulation.

[1] This *Journal*, xviii. 54. 1895.

THE REGULATION OF TEMPERATURE IN HYBERNATING ANIMALS.

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In a hybernating animal are present natural conditions, which are most favourable for the study of the regulation of temperature. During the winter sleep the activities of the tissues are reduced to a minimum and the animal resembles, as regards its temperature and the influence of changes in external temperature, the so-called cold-blooded animals. On the other hand, by arousing the animal from its torpor, it is possible to increase so greatly the activities of its body that its temperature rises rapidly and the animal in this active state is a warm-blooded animal, able to regulate its temperature.

The method we have employed to study the power of regulation of temperature is to determine the effect of sudden variations in external temperature upon the output of carbonic acid, the temperature and general activity of the animal. A detailed description of the method and results obtained upon various other animals has been previously given by one of us[1].

PART I. *Observations upon Hybernating Dormice.* By M. S. PEMBREY and W. HALE WHITE.

As is well known from the work of previous observers the temperature and metabolism of a hybernating animal are exceedingly low, and within certain limits vary with, and in the same direction as, the temperature of the surroundings. On these general points we may now give some of our observations; many additional experiments will be found in the Appendix to this paper.

[1] Pembrey. This *Journal*, xv. 401. 1893; xvii. 331. 1894, and xviii. 363. 1895.

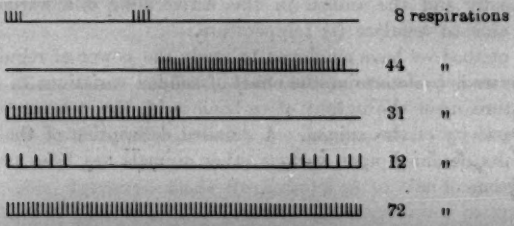
24. xii. 95. Observations showing that the temperature of a hybernating dormouse varies with that of the surrounding air.

Temp. of Air	Rectal temp. of Dormouse	Time	Remarks on Dormouse
5·5°	9·0°	3.40 p.m.	Coiled up, fast asleep.
10·0	11·0	3.55	" "
12·0	14·5	4.10	" "

As an instance of the low metabolism during hybernation we may quote an experiment in which we found that a dormouse in a quarter of an hour only discharged 14 decimgrms. of carbonic acid, whereas the same dormouse in an equal time on the same day discharged over 200 decimgrms. of carbonic acid when it was not hybernating.

In passing we may point out that, as other observers have noticed, when a hybernating animal is asleep its breathing is often of the Cheyne-Stokes type. The following observation shows this.

24. xii. 95. Temperature of the air of room = 6·75°. The dormouse No. X. is coiled up, its eyes are closed. The bulb of the thermometer is placed in the centre of the coil, T. = 9·5°. The respirations are of Cheyne-Stokes type and the following diagrams represent them in four consecutive minutes, and the last one eight minutes later.



When we study the effects of rapid changes in external temperature we see that the output of carbonic acid and the temperature of the animal vary according to the activity of the animal. If the dormouse is very active it responds to a fall in external temperature with increased muscular activity, and thus maintains the heat of its body, and indeed raises it somewhat.

Observations to show that if the dormouse maintains its temperature in spite of a fall in external temperature it is awake and active and the first effect of the fall in temperature is to raise that of the animal.

22. xii. 95. Large-sized female dormouse.

Temp. of Air	Rectal temp. of Dormouse	Time	Remarks on Dormouse
20·5°	32·5°	5.10 p.m.	Wide awake and very active.
18·5	32·25	5.25	" " "
7	36	5.45	" " "
7	34	6.0	" " "
7	33	6.30	" " "
6·5	33	7.0	" " "
6·5	31	7.30	" " "
6·0	34	8.30	" " "

The following shows that even apart from a rise in the external temperature the awaking of the animal causes its temperature to rise rapidly, twenty-two degrees in one hour.

Temp. of Air	Rectal temp. of Dormouse	Time	Remarks on Dormouse
9·25°	11·0°	7.0 p.m.	Fast asleep.
9·25	13·5	8.0	Not quite so fast asleep.
9·5	35·75	9.0	Wide awake. Running about.

If the animal is only fairly active a fall in external temperature generally causes it to become quiet, coil itself up and go to sleep; the output of carbonic acid and the temperature of the animal fall. Should the external temperature be suddenly raised to its previous height the output of carbonic acid still continues to fall and then increases gradually until the animal awakes, when there is a rapid and enormous increase in the carbonic acid. A sudden rise in the external temperature leads to a slow rise in that of the animal until it begins to awake, then its temperature suddenly mounts as much as fifteen degrees in a few minutes. These points are illustrated by the following experiments.

29. xii. 95. *Dormouse No.* 2. The fall of the animal's temperature, when it goes to sleep owing to a fall in the external temperature, is well illustrated by the following observations. The experiment also shows that there may be at first a small transitory rise in the creature's temperature, and that when it awakes its temperature runs up many degrees, even if the external temperature remains low.

Temp. of Air	Rectal temp. of Dormouse	Time	Remarks on Dormouse
21·25°	33·5°	12.15 p.m.	Wide awake, running about.
At 12.15 brought into a room, Temp. 9·5°.			
9·5	34·5	12.30	Wide awake, running about.
9·5	29·0	1.0	" " "
9·25	30·5	1.45	" " "
9·25	18	2.30	Coiled up and begins to go to sleep, although its eyes are open.
9·25	14·5	3.0	Coiled up asleep.
9·25	14·5	3.30	" "
10·0	14·5	4.30	" "
9·25	11·0	7.0	Very fast asleep, and motionless when temp. taken.
9·25	13·5	8.0	Slightly more awake.
9·5	35·75	9.0	Wide awake and running about.
10·0	33·5	11.15	Wide awake, eating.

Dormouse No. 1. 21. ix. 94. The active dormouse was in the ventilated chamber, water-bath = 25°, for 35 minutes before the first period. Temperature of the air of the room = 13°. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
25°	236	Active; washing itself.
23·75	173	Quiet.
25·5	188	Quiet; coiled up, apparently asleep.
15	183	Woke up for a moment, then quickly coiled itself up.
15	59	Quiet; coiled up.
24·25	23	" "
25	20	" "
24·5	159	Awakes towards the end of this period.
25	335	Awake but fairly quiet.

If the dormouse is in a sleepy, coiled-up condition a sudden fall in external temperature may at first arouse the animal and cause an increase in the discharge of carbonic acid, but in a few minutes the creature coils itself up and falls into a deep sleep, from which it is not readily aroused by a rise in the external temperature. If the dormouse does not wake up there is no sudden increase in the discharge of carbonic acid and the effect of the cold temperature persists.

Dormouse No. 1. 24. ix. 94. The sleepy dormouse was in the ventilated chamber, water-bath = 25°, for 30 minutes before the first period. Temperature of the air of room = 15°. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
25°	113	Coiled up.
24·75	112	"
15	220	Active at first, then coiled up.
15	68	Coiled up.
15	21	"
25	17	"
25	14	"
25	20	"
24·5	25	"
24·75	20	"

The dormouse was coiled up and fast asleep at the end of the experiment.

We have also repeatedly found that when the surrounding temperature is raised that of a hybernating dormouse is never much above it until it reaches a point at which the animal wakes, when its temperature rushes up many degrees in a few minutes.

It is therefore only within certain limits that the hybernating dormouse resembles a so-called cold-blooded animal, in that a rise or fall of external temperature produces a slow change in the same direction in the discharge of carbonic acid and the temperature of the animal. This effect moreover is more rapid than in the case of a frog.

When, as in the following experiment, the external temperature has been maintained at 30° for some time, the animal remains quiet and the discharge of carbonic acid is small. A sudden fall in the temperature of the water-bath to 22° quickly arouses the animal and with little or no delay the output of carbonic acid is doubled. In this respect the hybernating dormouse resembles a warm-blooded animal.

Dormouse No. 1. 25. ix. 94. The sleepy dormouse was in the ventilated chamber, water-bath = 30°, for 25 minutes before the first period. Temperature of the air of the room = 13·5°. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
30°	80	Quiet; coiled up.
30	88	Active for a few minutes.
30	73	Quiet.
22·5	163	Wakes up; active at times.
22·75	194	Active.
22·5	136	Active at times.

The weight of the dormouse was from 6 to 7 grms.

The experiment already quoted (p. 479) shows that, as regards the temperature also, when that of the air is lowered suddenly that of the animal rises if it remains awake.

The following observations show how a rise in external temperature increases the respiration, heart-beat, temperature and general activity of a hybernating animal.

Dormouse No. X. 26. xii. 95. 2.50 p.m. Dormouse asleep, coiled up. Temperature of air of room = 4° C. Respirations = 28 per minute, continuous type. Heart sounds cannot be heard with stethoscope. Temperature in coil of dormouse = 5°. 3 p.m. The dormouse was uncoiled and temperature taken in the rectum = 7°; dormouse moved during the process and made a noise when the bulb of the thermometer was inserted and removed, but it coiled itself up again; its eyes were not opened. Respirations are now deeper, 32, continuous type.

3.4 p.m. Temperature in coil of dormouse = 7·5°; two minutes later the respirations are 78 per minute, continuous type. The temperature in the coiled-up body is now 8°. Heart sounds cannot be heard.

3.10 p.m. The dormouse was brought into a warm room, temperature = 13°.

3.15 p.m. Temperature in coiled-up body = 13°. Respirations 140, continuous type.

3.21 p.m. Temperature = 17·5°; heart sounds can be distinctly heard, about 120 per minute.

3.27 p.m. Dormouse opens its eyes, temperature = 19°.

3.31 p.m. Dormouse walks about, temperature = 29°.

3.35 p.m. Temperature of the now active dormouse = 32°.

PART II. *Observations upon Hybernating Bats.* By M. S. PEMBREY.

In the case of these hybernating animals observations can be more readily made than on the dormouse, for the respirations are deeper and more readily counted and, since the body is not coiled up during sleep, the rectal temperature can be taken without greatly disturbing the animal.

The marked effect of activity upon the beat of the heart can be readily heard by means of a stethoscope or even by holding the animal close to the ear. I am indebted to Mr Leonard Hill for first demonstrating this to me and also for the gift of a bat, an animal which it is difficult to obtain in winter.

As in the case of other hybernating animals, so also in the bat there is a very great reduction in the activities of the tissues and the

discharge of carbonic acid; the temperature is low and varies with the external temperature; the respirations are few in number, shallow and often of the Cheyne-Stokes type; the heart beats slowly and feebly and no sound can be heard with the stethoscope.

Bat II. 29. xii. 95. Temperature of air of room = 10·75°. Bat quiet, respirations 13 per minute, Cheyne-Stokes breathing well marked. Two minutes later the bat moves slightly and opens its eyes. Heart sounds cannot be heard. Respirations 40 per minute. Rectal temperature = 11·5°.

Bat II. 4. ii. 96. Temperature of air of room = 11°. Bat is asleep, its rectal temperature = 11°. Respirations of continuous type, 35 per minute. The bat was in the ventilated chamber, water-bath = 10·9°, for 30 minutes before the first period. Consecutive periods of 1 hour.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat
10·9°	16	Quiet.
10·9	17	"
11	15	"
11	15	"
11	16	"

The bat is very inactive; its rectal temperature at the end of the experiment is 12°; respirations are 40 per minute, continuous in type.

Bat II. 25. xii. 95. Bat quiet, eyes closed. Temperature of air = 3°. Heart sounds cannot be heard. Respirations 12 per minute, Cheyne-Stokes type. Rectal temperature = 2·25°.

If a hybernating bat be brought from a cold room into a warm one, its temperature begins to rise gradually until it awakes and moves about, when there is a sudden and rapid rise far above the temperature of the room.

Bat II. 24. xii. 95. Temperature of the air of room = 6·5°. The bat is inactive, but moves slightly at times; its eyes are closed. 3.35 p.m. Rectal temperature of bat = 6·75°; respirations = 60 per minute, continuous type; heart sounds cannot be heard.

3.41 p.m. Bat was brought into a warm room (11·5°). Respirations = 100, continuous type; heart sounds cannot be heard.

3.52 p.m. Rectal temperature = 11·25°; a minute or two later the respirations are 160 per minute, continuous type, and the heart sounds can be distinctly heard, rate seems to be 160 per minute.

4 p.m. The bat begins to move and opens its eyes.

4.6 p.m. Rectal temperature = 33·5°. The bat is very active, washes itself. The heart sounds are very loud, but are too rapid for counting.

The exposure of an active bat to a low external temperature greatly increases its activity and raises its temperature.

Bat II. 17. xii. 95. The bat had been much disturbed and was very active. It was placed in a beaker surrounded by ice and water, the bat became more and more active and its temperature rose, the respirations became more rapid and the heart sounds very loud.

When the inactive bat is subjected to sudden changes of temperature, it responds in a somewhat similar way to that seen in a cold-blooded animal; a rise or fall in external temperature determines changes in the same direction in the output of carbonic acid, the temperature and activity of the animal. The response, however, is more rapid than in the case of a cold-blooded animal such as the frog[1], and resembles that observed in an immature mouse or pigeon[2].

In the following experiment is shown the effect of a sudden change in the external temperature from 13° to 25°.

Bat II. 2. i. 96. The temperature of the animal, respirations &c. were first determined and then the bat was kept in the ventilated chamber, water-bath = 12·5°, for 1¼ hours before the first period. Consecutive periods of 30 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds
(Air = 14·75°)		Inactive	15·5°	120 continuous type	Cannot be heard.
12·5	16	Quiet			
12·75	13	,,			
13	6	,,			
25	47	,,			
25	56	Asleep	25	180 (approx.)	Very loud; too rapid for counting.

Should, on the other hand, the rise of temperature awake the bat and make it active, then there is seen, as in the case of the dormouse, a sudden and, one might say, an explosive discharge of carbonic acid.

Bat II. 3. i. 96. The temperature of the animal, respirations &c. were first taken and then the bat was kept in the ventilated chamber, water-bath = 14°, for 45 minutes before the first period. Consecutive periods of 30 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds
(Air = 13·5°)		Asleep	16·5°	10 Cheyne-Stokes type	Cannot be heard.
13·75	20	Quiet			
13·75	18	,,			
30	38	,,			
30	83	,,			
29·5	444	Very active			
26	451	,, ,,	30	Too rapid for counting	Too rapid for counting. Very loud.

[1] Pembrey. *Proc. Physiol. Soc.* This *Journal*, XVI. 1894.

[2] Pembrey. This *Journal*, XVIII. 363. 1895.

The above effect is not due to the warm external temperature but to the muscular activity of the awakened animal, for if an inactive bat be forcibly aroused there is a similar sudden increase in the output of carbonic acid, although the temperature of the surroundings remains low. This condition is shown in the next experiment.

Bat II. 4. i. 96. The temperature of the animal, respirations &c. were first taken and then the bat was kept in the ventilated chamber, water-bath = 14·5°, for 5 minutes before the first period of 30 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds	
(Air = 13°)		Quiet	15·5°	18 Cheyne-Stokes type	Cannot be heard.	
14·25°	20	,,	15·75	6 Cheyne-Stokes type	,,	,,

The bat was now placed on its back several times and made active. In a minute or two the heart sounds could be distinctly heard and the respirations became rapid. Within 14 minutes the rectal temperature rose to 19°. The bat was very active and was placed in the ventilated chamber, water-bath = 14°, for 5 minutes before the next period of 30 minutes.

14°	126	Active	18°	57	Loud.

Muscular activity is here shown to rapidly increase the temperature of the animal and the discharge of carbonic acid. The temperature of the bat may be raised to 25° by an external temperature of 25°, but if the animal remains asleep the output of carbonic acid is about one half (56 decimgrms., see Expt. 2. i. 96), the amount resulting from the activity of the awakened animal (126 decimgrms.). This takes place even although the rectal temperature of the awakened bat is only raised to 18° and the external temperature remains at 14°.

The muscular activity of the animal is the important factor in increasing the production of heat. In this respect the following determination of the weights of the muscles and glands in bat No. II. is of interest.

The weight of the bat		= 19·94 grms.	
,,	,, voluntary muscles	= 6·13	,,
,,	,, heart	= 0·248	,,
,,	,, liver, kidneys, spleen and salivary glands	= 0·984	,,
,,	,, stomach & intestines (contents washed out)	= 0·860	,,

There was a small quantity of fat in the mesentery, but little or none in the subcutaneous tissue. No disease could be found. There was a small

quantity of food in the stomach and intestines; the bladder contained a little urine. During the winter the bat became somewhat thin, but the loss was not very marked. When very active it would sometimes eat a very small quantity of bread and milk, but would not eat meat.

Conclusions.

It seems to both of us that the most important point to be deduced from all these experiments is that the temperature of a hybernating animal depends upon its muscular activity far more than upon the temperature of the air, indeed a low external temperature cannot reduce the animal's temperature unless it first causes its muscular activity to diminish. Our experiments also show that in these animals the regulation of temperature is chiefly effected by alterations in the production of heat, for the rise of temperature was always accompanied by an increased output of carbonic acid; this was especially shown in the almost explosive discharge of carbonic acid which takes place when the animal awakes from its hybernating condition and its temperature mounts many degrees in a few minutes.

The enormous and sudden increase in the output of carbonic acid and the greatly increased activity of the animal, when its temperature rises, point to the fact that the chief cause of this rise is to be sought in the increased metabolism of the muscles. Diminished loss of heat plays little or no part in this process for the surface is more vascular when the animal is active. Further, if the animal begins to hybernate and the external temperature is falling its internal temperature and its output of carbonic acid fall very quickly and the animal is very inactive. It thus appears that the hybernating animal has little power of maintaining its temperature by diminishing the loss of heat, except in so far as the dormouse attempts to economize by coiling itself up and ruffling its fur.

The increase in the force and frequency of the heart's beat when the animal awakes would by the heat arising from friction in the vessels also tend to raise the temperature; likewise the quickened respiration and movements of the limbs would produce a similar result. The heat, however, arising from friction in these cases would be unimportant compared with the heat produced by the processes of combustion, which accompany contraction of the voluntary muscles. It is one of the important functions of voluntary muscles to produce heat, but from the

scanty data existing it is probable that the contraction of the cardiac muscle is, from a physical point of view, a much more economical process, work and not the production of heat being its function.

The hybernating animal is in many respects comparable to an anæsthetised mouse[1], and it is possible that its production of carbonic acid and its temperature vary with the external temperature, because sensory impulses from the skin and muscles are diminished and thus reflexly less metabolism occurs in the muscles.

The power of regulation of temperature observed in the hybernating dormouse and bat resembles closely that of an immature warm-blooded animal[2], and as regards the evolution of hybernation we might say that it is not due to the acquisition of a new power, but to the retention of one already present in immature mammals and birds[3].

Previous Work upon the Subject.

Although a large number of observations and experiments have been made upon hybernating animals, there are, as far as we can ascertain, no experiments similar to our own. The method employed in our experiments tests the power of the animal to maintain its temperature when the external temperature is suddenly changed.

We shall consider here only the previous work which bears directly upon our own, and give in the "References" a list of the most important papers upon the subject of hybernation.

John Hunter about the year 1766 showed that the exposure of dormice to the cold of a freezing mixture increased the activity and raised the temperature of the animals. In several cases the animal soon died.

Spallanzani noticed that during hybernation the respiration was so slight that marmots and bats could be kept for four hours in carbonic acid gas without suffering any ill effects, whereas a bird and a rat placed in the chamber at the same time died at once.

Saissy published in 1808 a series of experiments, in some of which he had determined the intake of oxygen by dormice under different conditions; he found that the amount of oxygen used varied as the activity of the animal, and that during well marked hybernation there

[1] Pembrey. *Proc. Physiol. Soc.* This *Journal*, 1895.
[2] Pembrey. This *Journal*, XVIII. 363. 1895.
[3] Hale White. *Journ. of Anat. and Phys.* XXV. 374.

was hardly any intake of oxygen. Saissy found that exposure to cold alone would not cause an active marmot to hybernate, but by means of prolonged exposure to a temperature below zero and greatly diminished ventilation he was able to make a marmot hybernate in summer. The temperature of this animal fell from 35° to 5° and, although the experiment was repeated, no bad effects could be observed.

Edwards showed that bats when exposed to an external temperature of 1° did not maintain their temperature, and that in this respect they resembled immature warm-blooded animals. He concluded that bats under all conditions produce less heat than ordinary warm-blooded animals.

Marshall Hall made experiments upon dormice and bats and in some cases determined by means of his pneumatometer the intake of oxygen under different conditions. Thus he found that a "bat, which in a state of hybernation passed ten hours without respiration, absorbed or converted 5·8 cubic inches of oxygen gas into carbonic acid in one hour, when in a state of activity. In an intermediate condition, it removed one cubic inch of oxygen in two hours and forty minutes." Marshall Hall attempts to draw a distinction between hybernation and a condition which he terms "torpor"; thus in opposition to all observers he maintains that in the first condition the sensibility of the animal is not impaired.

Regnault and Reiset determined the respiratory exchange of several hybernating marmots and found that the intake of oxygen was about $\frac{1}{30}$th of that of an active animal and only about $\frac{3}{4}$ths of the oxygen appeared in the carbonic acid discharged. The following are two examples:—

Condition of Marmot	Grms. per Kilo and hour: O_2 intake	CO_2 output	$\frac{CO_2}{O_2}$
Hybernating	0·48	0·037	·588
Awake	1·198	1·312	·796

A further proof that oxygen was stored up in the body of the hybernating animal was found in the increase in weight of a marmot during profound torpidity; it gained as much as 5·9 grms in five days.

Similar results have been obtained by Valentin and other observers.

Horvath between the years 1877 and 1881 published numerous observations upon two or three species of spermophiles, and marmots, hamsters, hedgehogs, dormice and bats. Determinations of the output of carbonic acid and the intake of oxygen were made in the case of some

spermophiles and dormice. Horvath found that in the hybernating condition the respiratory exchange was very low [CO_2 = ·025 grm. in three hours, rectal temperature of spermophile = 9°], whereas it was high when the animal was active [CO_2 = ·457 grm. in half-an-hour, rectal temperature = 33·5°]. Similar results were found in the case of dormice. The determinations were made by analyses of the air taken at different times from a closed bell-jar in which the animal was kept. Horvath himself pointed out the unsatisfactory nature of such a form of respiration apparatus and in many cases gave only the percentages of oxygen and carbonic acid because the volume of the animal was unknown. He also remarks that in some cases the percentage of oxygen was considerably over 21 per cent. In his own words "diese Gasanalysen sind nur als eine Voruntersuchung zu betrachten." Notwithstanding these sources of error Horvath's work was most important, for he tried to find out if the sudden rise in the temperature of the hybernating animal could be accounted for by a sudden increase in combustion; he showed that it was probable but refused to draw any conclusion.

Numerous physiologists have made observations upon the temperature, respiration and heart-beat of hybernating animals and have shown that cold alone will not cause hybernation, when the animal is active. Valentin and Horvath give cases of hybernation during the summer, but the cause could not be ascertained.

Hale White in a paper describing experiments in which dormice were used to determine the specific heat of living animal tissues gives a number of observations on their temperature which accord with the results now published.

Preliminary communications of this research were given at the meetings of the Physiological Society, Oxford, June 29th, 1895[1], and Charing Cross, January 11th, 1896.

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APPENDIX.

In this Appendix are placed the notes of additional experiments.

PART I. *Observations on Dormice.*

22. xii. 1895. Male Dormouse.

Temp. of air	Rectal T. of mouse	Time p.m.	Remarks
5·5°	9°	3.40	Quiet, coiled up.
10	11	3.55	,, ,,
12	14·5	4.10	,, ,,
18	26	4.25	Awake.
17	28	4.40	,,
20	33	4.55	,,
20·5	33	5.10	,,
18·5	33·25	5.25	,,
7	30	5.45	Very lively.
7	31	6.0	,,
7	31	6.30	,,
6·5	29	7.0	,,
6·5	30·5	7.30	,,
6	34	8.30	,,

22. xii. 1895. Female Dormouse.

Temp. of air	Rectal T. of mouse	Time p.m.	Remarks
5·5°	9°	3.40	Quiet, coiled up.
10	10·5	3.55	,, ,,
12	14·25	4.10	,, ,,
18	18·5	4.25	Awake.
17	23	4.40	,,
20	30	4.55	,,
20·5	32·5	5.10	,,
18·5	32·25	5.25	,,
7	36·0	5.45	Very lively.
7	34	6.0	,,
7	33	6.30	,,
6·5	33	7.0	,,
6·5	31	7.30	,,
6·0	34	8.30	,,

23. xii. 1895. Male Dormouse.

Temp. of air	Rectal T. of mouse	Time p.m.	Remarks
15°	34°	6.30	Wide awake, lively.
22	35	7.0	,, ,,
27	36·5	7.30	,, ,,
17	33·0	9.45	,, ,,
7	33·5	10.15	,, ,,
7	32	10.45	,, ,,
7	31·5	11.15	,, ,,

23. xii. 1895. Female Dormouse.

Temp. of air	Rectal T. of mouse	Time p.m.	Remarks
15°	31°	6.30	Wide awake, lively.
22	35	7.0	,, ,,
27	36·5	7.30	,, ,,
17	33	9.45	,, ,,
7	36	10.15	,, ,,
7	32	10.45	,, ,,
7	32	11.15	,, ,,

29. xii. 1895. Male Dormouse.

Temp. of air	Rectal T. of mouse	Time	Remarks
9·5°	10°	11.15 a.m.	Fast asleep, tightly coiled up.
21·5	18	11.30	Just begun to wake, breathing fast, opened eyes.
22·0	26·25	11.45	Fully awake, running about, respirations 180.
23	34	12.0	Wide awake, running about.
21·25	33·25	12.15 p.m.	,, ,, ,,
9·5	34·0	12.30	,, ,, ,,
9·0	32·25	1.0	,, ,, ,,
9·25	31	1.45	,, ,, ,,
9·25	30·5	2.30	,, ,, ,,
9·25	28	3.0	Rather sleepy.
9·25	34	3.30	Wide awake, having been roused by taking T. at 3.
10·0	32	4.30	Wide awake.
9·25	14·5	7.0	Very sleepy, does not resist taking T. but moves its legs, opens its eyes and is not completely coiled up.
9·25	36·0	8.0	Wide awake and running about.
9·5	34·5	9.0	,, ,, ,,
10	33·0	11.15	,, ,, ,,

29. xii. 1895. Female Dormouse.

Temp. of air	Rectal T. of mouse	Time	Remarks
9·5°	10°	11.15 a.m.	Fast asleep, tightly coiled up.
20	16	11.30	Waked a little but not so much as male mouse, opened its eyes. At 11.35 wide awake on its feet.
20	20·5	11.45	Not so wide awake as male, for although on its feet does not run about.
21·75	29·0	12.0	Wide awake and running about.
21·25	33·5	12.15 p.m.	,, ,, ,,
9·5	34·5	12.30	,, ,, ,,
9·5	29·0	1.0	,, ,, ,,
9·25	30·5	1.45	,, ,, ,,
9·25	18·0	2.30	Coiled up, begun to sleep, eyes still open.
9·25	14·5	3.0	Coiled up, fast asleep, eyes shut.
9·25	14·5	3.30	,, ,, ,,
10	14·5	4.30	,, ,, ,,
9·25	11·0	7.0	Coiled up, very fast asleep.
9·25	13·5	8.0	Not quite so fast asleep as last note.
9·5	35·75	9.0	Wide awake, running about.
10·0	33·5	11.15	,, ,, ,,

3. i. 1896. Male Dormouse.

Temp of air	Rectal T. of mouse	Time	Remarks
9·75°	10·5°	12.10 p.m.	Fast asleep, eyes shut, tightly coiled up.
15·0	12·75	12.25	Moving limbs, but eyes still shut.
15·0	14·75	12.40	,, ,, ,,
15·0	16·0	12.55	,, ,, ,,
16·5	17·0	1.10	Rather more awake.
27	22·5	1.25	Almost wide awake.
28	30	1.40	Wide awake.
23·5	34·75	1.55	,,
24·0	34·0	2.10	,,

3. i. 1896. Female Mouse.

Temp. of air	Rectal T. of mouse	Time	Remarks
9·75°	10·5°	12.10 p.m.	Fast asleep, eyes shut, tightly coiled up.
15·0	12·0	12.25	" " "
15	14·0	12.40	Fast asleep, has opened eyes & moved a little.
15·25	15·0	12.55	" " " "
16·0	16·0	1.10	Rather wider awake.
27·0	21·0	1.25	" "
23	30	1.40	Wide awake.
23·5	33	1.55	"
24·0	32·25	2.10	"

5. i. 1896. Male Dormouse.

Temp. of air	Rectal T. of mouse	Time	Remarks
16°	16·5°	5.0 p.m.	Fast asleep, tightly coiled up.
25	21·0	5.15	Partially awake, moving limbs.
25·5	23·5	5.30	" "
25	29	5.45	Wide awake.
26·5	34·5	6.0	"
8·0	24·0	8.30	Lethargic and beginning to go to sleep. Gave the animal some food.
8·0	34·5	11.0	Wide awake, has made a hearty meal.

5. i. 1896. Female Dormouse.

Temp. of air	Rectal T. of mouse	Time	Remarks
16°	17°	5.0 p.m.	Fast asleep, tightly coiled up.
25	20·5	5.15	Partially awake, moving limbs.
25·5	27·5	5.30	Wide awake, running about.
25·0	35	5.45	" " "
26·5	35·5	6.0	" " "
8·0	25·0	8.30	Lethargic and beginning to go to sleep. Gave some food.
8·0	36·5	11.0	Wide awake, has made a hearty meal.

Dormouse X.

Date	Temp. of air	Temp. of Dormouse	Time	Remarks on the Dormouse
23. xii. 95	13·5°	37°	1.10 p.m.	Very active.
24. xii. 95	6·5	31	3.30	Awake, but listless. Heart sounds can be heard distinctly.
25. xii. 95	4·5	25	11.58 a.m.	Awake, but listless.
26. xii. 95	.4	5	2.50 p.m.	Asleep, coiled up. Respirations 28 per min. Heart sounds cannot be heard.
	4	7·5	3.8	Slightly disturbed by taking temperature. Respirations 78. Heart sounds cannot be heard.
	13	13	3.15	Respirations 140.
	13	17·5	3.21	Heart sounds can be heard.
	13	19	3.25	Awakes. Heart sounds can be heard distinctly.
	13	29	3.32	Moves about.
	13	32	3.35	Active.

Dormouse Y.

Date	Temp. of air	Temp. of Dormouse	Time	Remarks on the Dormouse
30. xi. 95	17°	30° Rectal 31 Bulb in coiled-up body	2.15 p.m.	Inactive.
2. xii. 95	12	13·75 Rectal 14·25 Bulb in coiled-up body	11.25 a.m.	Hybernating.
3. xii. 95	15	19·5 Rectal 19·75 Bulb in coiled-up body	1.25 p.m.	Very inactive.

Dormouse, No. 1. 21. ix. 94. Dormouse active; temperature of air = 18°. The dormouse was in the ventilated chamber, water-bath = 25°, for 15 minutes before the first period. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
25°	197	Active; washing itself.
24·5	182	Quiet; goes to sleep.
15	146	Wakes up, then goes to sleep.
15	35	Asleep.
25·5	23	Quiet; asleep.
25	220	Asleep (?).
24·5	307	Very active at the end of the experiment.

Dormouse, No. 1. 26. ix. 94. Dormouse active; temperature of air = 18°. The dormouse was in the ventilated chamber, water-bath = 30°, for 25 minutes before the first period. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
30°	63	Quiet.
30	82	,,
30	105	,,
22	178	Very active.
22	183	Active.
21	182	Very active.

Dormouse, No. 2. 2. x. 94. Dormouse sleepy; temperature of air = 14°. The dormouse was in the ventilated chamber, water-bath = 25°, for 25 minutes before the first period. Consecutive periods of 15 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Dormouse
25°	78	Active at times.
25	77	,, ,,
15	90	Very active for 5 mins., then coiled up.
15	65	Quiet; coiled up.
25	35	Asleep; coiled up.

PART II. *Observations on Bats.*

Bat I. 13. xii. 94. The bat was in the ventilated chamber, water-bath = 20°, for 30 minutes before the first period. The bat was very active when placed in the chamber. Consecutive periods of 15 minutes.

Period	Temp. of water-bath	CO_2 discharged in decimgrms.	Period	Temp. of water-bath	CO_2 discharged in decimgrms.
1	20°	135	5	15°25	113
2	20·25	110	6	15·25	143
3	19·75	110	7	20	117
4	20	130	8	19·75	112

Bat I. 14. xii. 94. The bat was in the ventilated chamber, water-bath = 15·5°, for 15 minutes before the first period. The bat is very inactive. The temperature of the air of the room was 14°. Consecutive periods of 15 minutes.

Period	Temp. of water-bath	CO_2 discharged in decimgrms.	Period	Temp. of water-bath	CO_2 discharged in decimgrms.
1	15·5°	14	5	24·5°	14
2	15·25	10	6	24·5	20
3	15·5	11	7	25	29
4	25	11	8	25	30

The bat was very inactive at the end of the experiment.

Bat II. 20. xii. 95. The bat is inactive; respirations of Cheyne-Stokes type, 40 per minute; heart sounds cannot be heard. Rectal temperature was 12·25°. Temperature of air of room = 11·5°. The bat was in the ventilated chamber, water-bath = 11·5°, for 50 minutes before the period of 20 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.
11·25°	20

Bat II. 15. i. 96. 4.40 p.m. The bat is very active at times, although it has not been disturbed. The temperature, respirations &c. were first taken and then the bat was kept in the ventilated chamber, water-bath = 16°, for 5 mins. before the period of 30 mins.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds
(Air = 15·5°)		Active	21°	120 continuous type	Loud.
16	304	Very active	22	,,	,,

Bat II. 22. i. 96. The bat is quiet, its eyes are open, at times it crawls slowly. The temperature, respirations &c. were first taken and then the bat was kept in the ventilated chamber, water-bath = 14·5°, for 5 minutes before the first period of 30 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds.
(Air = 15°)		Inactive	16·25°	50 Cheyne-Stokes type	Cannot be heard.
14	72	Moves slightly at times	17		,, ,,

The bat was now placed upon its back several times and made to struggle; it was not handled. Respirations became very rapid; the heart sounds are very loud. Rectal temperature has risen in 12 minutes from 17° to 24°. The bat was very active and was placed in the ventilated chamber, water-bath = 14°, for 5 minutes before the next period of 30 minutes.

Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat	Rectal temp.	Respirations. No. per min.	Heart Sounds
14°	274	Very active at times	21°	Too rapid for counting	Loud

Bat II. 13. ii. 96. The bat is awake and fairly active. It is somewhat thin. Rectal temperature = 14·25°. Respirations 40 per minute, irregular. Heart sounds cannot be heard. Temperature of air of room = 14°. The bat was in the ventilated chamber, water-bath = 12°, for 35 minutes before the first period. Consecutive periods.

Time	Temp. of water-bath	CO_2 discharged in decimgrms.	Remarks on the Bat
1 hour	12·5°	26	Quiet.
2 hours	25	266	Moves slightly at times.

At the end of the experiment the rectal temperature was 24·25°. Respirations 100 per minute. Heart sounds cannot be heard.

Bat II. 19. xii. 95. Bat not very active. Temperature of air = 10·5°. Respirations of bat 11 per minute, Cheyne-Stokes type. Rectal temperature = 17°. Heart sounds cannot be heard. An attempt was now made to look at the blood vessels in the wing under the microscope. The bat resisted vigorously; the respirations became so rapid that they could not be counted, and the heart sounds were very loud; the rectal temperature was raised to 34°, that is a rise of 17 degrees within 15 minutes. The bat was left undisturbed for about 2 hours; at the end of this time it was less active, its respirations were 60 per minute and the heart sounds could not be heard. The rectal temperature was 15·75°. Temperature of air = 12°. The bat was now forced to move about and within 13 minutes the rectal temperature rose to 24°, and the heart sounds could be heard distinctly.

Bat II. 23. xii. 95. 3 p.m. Bat very inactive, eyes closed. Rectal temperature = 5·75°; temperature of air = 5°. The heart sounds cannot be heard. Respirations were 53 per minute after the animal was disturbed by the taking of the rectal temperature.

Bat II. 24. xii. 95. 1.30 p.m. Bat moves slightly at times. Respirations = 22 per minute, then 10 per minute, then 5 per minute, Cheyne-Stokes type. Heart sounds cannot be heard. Rectal temperature = 6·25°; temperature of air = 6·5°.

Bat II. 30. i. 96. 1 p.m. Bat slightly active. Respirations about 50 per minute; heart sounds cannot be heard. Rectal temperature = 16°; temperature of air = 15°. The bat was kept in ventilated chamber, water-bath = 15°, for 3 hours. Rectal temperature at the end of the experiment was 17·5°.

THE CHANGES IN SHAPE AND IN POSITION OF THE HEART DURING THE CARDIAC CYCLE.

By JOHN BERRY HAYCRAFT, M.D., and D. R. PATERSON, M.D. (Five Figures in Text.)

(*From the Physiological Laboratory, University College, Cardiff.*)

1. Changes in Shape.

The rapidity and complexity of the heart's action have always proved a difficulty in a study of the cardiac movements by the naked eye, and to overcome this various methods, graphic and other, have been employed. One of the most important is that devised by Ludwig and Hesse[1] in their well-known researches upon the movements of the heart. In order to obtain the heart in diastole they distended the ventricles of a still living heart by a pressure equal to the mean blood-pressure during life, and they plunged a similar heart emptied of blood into a hot saturated solution of bichromate of potassium at 80° C., causing it to contract and become fixed in the systolic condition. Plaster casts were taken of these two states and careful measurements of them were made. They found that both the front to back and side to side diameters were diminished during systole. The great objection to this method is that the heart is taken out of the chest, deprived of its natural supports, the lungs, and as it is not a rigid body it may suffer distortions both from manipulation and from the influence of gravity, and the shape of the cast so obtained may not be the shape of the organ in the unopened chest cavity.

It is however generally admitted that during systole the heart diminishes in the base to apex diameter, though to a slight extent, and also from side to side. But many observers refuse to accept the evidence of Ludwig's casts as bearing upon the change in the front to back diameter. Thus amongst many others, Landois[2] considers that

[1] Ludwig and Hesse. *Archiv f. Anat. u. Physiol.* 1880.

[2] Landois and Stirling. *A Text-book of Human Physiology*, 64.

the front to back diameter increases during systole and causes the upstroke of the cardiogram. The same view is maintained by Roy and Adami. They say[1]: "Under normal conditions when the chest wall is intact, the pressure both anteriorly and posteriorly of the chest wall and diaphragm respectively, causes the transverse diameter to be the more considerable. In systole, on the other hand, with the contractions of the ventricle, the walls become tense and resisting, the organs become rounded, the transverse diameter is diminished, the antero-posterior diameter increased."

In a former paper[2], one of us showed that if a balanced lever be placed upon the base of the exposed heart of a frog placed under the influence of ether and with the chest opened without loss of blood, the lever is elevated as the heart increases in front to back diameter during diastole, and falls as the heart diminishes during systole,—the reverse, in fact, of what we find in the excised or bloodless heart of the pithed frog. The same facts may be observed, and all the changes in the heart can be recorded, by viewing an enlarged picture of the heart through a glass ruled into equal squares and marking point by point on a piece of paper ruled in similar squares the changes which take place as the heart passes from systole to diastole. In the dog with an opened chest we can learn by inspection that the heart decreases in all dimensions during systole provided we are careful to inflate the lungs so as to buoy up the heart into its natural position. In order to determine the change in the front to back diameter of the heart in the unopened thorax, two needles were thrust into the left side of the chest of the rabbit parallel to each other and horizontal in direction. The upper one pierced the heart laterally and to the anterior surface, the lower one also laterally but to the posterior surface. Light straws were attached to the heads of the needles projecting outside the chest and the straws moving away from each other indicated that at each systole, the needles inside the chest were approximated.

It recently occurred to us that the question might be tested by the use of another method. This was to kill one animal with the heart in diastole and another with the heart in systole, and make frozen sections of the undisturbed thoraxes, with the hearts *in situ*, so that any difference between the front to back diameters could be determined. We

[1] Roy and Adami. *The Practitioner*, XLIV. 173.

[2] This *Journal*, XII. 447. 1891.

experienced great difficulty in killing an animal so that the heart died in systole. Strophanthine, caffeine, digitaline, barium chloride, which are so active in the frog gave in the case of the dog most unsatisfactory results, for the left ventricle was seldom properly contracted whilst the right was often widely distended. The most satisfactory results were obtained by injecting a saturated solution of corrosive sublimate into the right jugular vein, the heart ceasing in systole almost immediately. The dogs experimented on were placed on the back and received the lethal injection after administration of an anæsthetic. After death the bodies were allowed to pass into rigor mortis, and, when stiff, the head and limbs were severed, and the trunk placed in a crate of wood made to fit. This was covered by india-rubber sheeting to keep the parts perfectly dry, and the whole was placed in a large box, and well packed with ice and salt. After about forty-eight to seventy-two hours, the crate was opened and sections were made of the frozen trunk by help of a fine saw. The sections were transverse and serial, and were commenced below. When the area of the heart was reached, each transverse section was washed clean with methylated spirit and photographed. By means of the sections preserved in alcohol and the photographs made from them it was possible to measure accurately not only the size but also the position of the heart in the chest cavity. In these experiments, it was necessary in order to compare the contracted heart of dog *A* with the dilated heart of dog *B*, either that the dogs should be identical in size or that a correct allowance should be made for any difference. This correction might perhaps have been made in terms of the weights of the two dogs, but here an error occurs from varying weights of fat, not necessarily associated with variations in the size of the heart. In preference to this method we took the mean of the inside greatest transverse diameter of the chest and of the front-to-back diameter, measured from the back of the sternum to the front of the vertebra, both taken at the level of the middle of the ventricles. The number thus obtained say 8·5 centimetres was multiplied by 1·176, bringing it to 10 centimetres, and all the measurements of the heart were likewise multiplied by the same number (1·176). In the case of a smaller dog where the mean diameter was, let us say 5, all the heart's measurements would have to be multiplied by 2. In this way all our measurements can be expressed in terms of a standard dog with a mean chest diameter of 10 centimetres. We found this principle to work out with very fair accuracy, for, on comparing measurements which had been

standardised from dogs of various sizes, all of them with the heart in dilatation, the numbers closely approximated and were amply sufficient for practical purposes.

In the various dogs from which measurements were taken, we made use of the photographs and the hardened sections which had been preserved in alcohol. We were not infrequently met with the difficulty of obtaining corresponding points on the photographs. This was to some extent overcome by having recourse to the sections, and in all cases these were carefully compared and standardised in the way mentioned. Even then there remained the difficulty of obtaining absolutely accurate figures on which to base a comparison. This objection of course applies equally to measurements taken from casts, as well as by other methods, for we cannot obtain fixed points on the heart, and consequently our measurements cannot be as absolute as those, say, of the length of a bone.

In the subjoined table we give the measurements obtained from two dogs, one in which the heart was in a state of diastole and the other which showed the organ in systole.

Heart of Dog. Measurements taken through the middle of the Ventricles.

	Dilated	Contracted
Side to side	6·32 cms.	5·31 cms.
Front to back	5·70 „	4·72 „

Mean diameter of chest—10 cms.

These figures taken from sections through the middle of the ventricles indicate a diminution of both diameters in about the same proportion during systole.

Another point brought out by an examination of these sections is that the heart has on transverse section a more or less circular outline both in systole and diastole. This form is usually represented in books as more or less flattened from before backwards during diastole, and is said to approach closely to that of an ellipse. When a bloodless and excised heart is placed upon a table it flattens upon the table during diastole. If the organ is placed upon its posterior surface the side to side diameter is increased; if it is laid on its lateral surface the front to back diameter is increased. Our sections show that these effects of gravity are but slight and unimportant within the chest cavity for here we have the organ enclosed and supported by the lungs.

In Fig. 1, a tracing is given of a photograph taken through the middle of the heart of a dog killed with its heart in diastole. The section was photographed from below and the left ventricle therefore appears on the right hand of the drawing (*L*). Fig. 2 is a tracing from a photograph of a section taken through the thorax of a dog killed by corrosive sublimate. The figures given in the table on page 499 were

Fig. 1.

Fig. 2.

taken from these sections approximately at the level photographed. In Fig. 2 there is some effusion into the pericardium, due possibly to the irritation of the corrosive sublimate, which had passed from the jugular into the right ventricle. Inspection will indicate what the measurements clearly show, namely, that both in the contracted and dilated heart the side to side diameter is slightly greater than the front to back diameter, the greatest diameter being oblique, and running from behind, forwards and to the right. There is therefore no change from an elliptical into a circular transverse diameter as described by some. It is evident too that the chief change is one of size not of shape, the heart in Fig. 2 occupying a smaller area proportionally to the chest cavity than the heart of Fig. 1.

II. Changes in Position.

Since Harvey's time physiologists have held that during systole the heart changes its position in the chest cavity. The heart is supposed to twist on its long axis so that during systole the left side comes more to the front, the heart and especially the apex is supposed to spring forwards and to the right. Harvey described these changes very graphically, and one can observe them as he observed them in the opened chest of any animal lying on its back. As one of us in the paper already referred to pointed out, an animal is invariably placed on its back and the chest opened in front, for it is the easiest position for observation. If however the animal be suspended in the air with the front of the chest downwards, and if the chest be then opened, the heart may be observed to move towards the sternum during diastole and spring towards the vertebral column during systole. If the animal be placed on one side the heart always sinks towards that side during diastole and springs to the other side during systole. The heart in point of fact becomes during diastole so flaccid that it sinks under gravity in whatever position it is placed, and only during systole does it succeed in asserting itself against gravity.

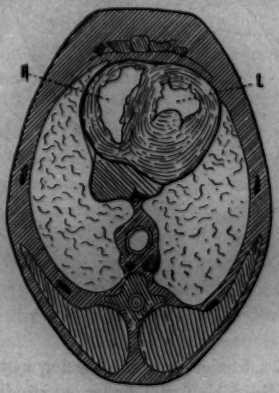

Fig. 3.

When the animal is placed upon the back and the chest opened the heart falls towards the back, and owing to anatomical arrangements,

such as the great bulk of the liver being on the right side, it inclines to the left. This gravity action takes place during diastole when the heart is flaccid, and the recovery from this position brings the heart towards the sternum with some rotation back to the right. When we assume that the same change occurs in the unopened chest we do so without warrant, for the two cases are very different. In the opened chest the heart is no longer supported on all sides and it sinks with gravity. Not only so but it is free during systole to regain its former position. In the unopened chest the heart is surrounded by the lungs, and if gravity displaces it this will be to a smaller degree depending upon its specific weight being in excess of the lungs themselves. Not only so but the heart in changing its position in the chest has to overcome the resistance of the lungs. Thus if a person lies prone upon the back the heart sinks but slightly, for the lungs have to be so deformed in shape that they make room for it below and close over it towards the sternum. In

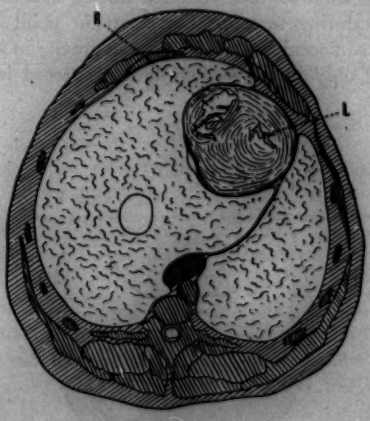

FIG. 4.

Fig. 4, a tracing from a frozen section of a dog killed with the heart in diastole, it will be noticed that the heart which is cut through at the lower third of the ventricle is almost entirely on the left side. The body was placed on that side immediately after death and the heart has sunk under the influence of gravity. In doing so it has had to compress

the left lung and the right lung has closed over and enveloped it. The action of gravity in displacing the heart is therefore quite apparent in the unopened chest. It is still an important factor and notoriously shifts the cardiac impulse when a patient lies upon his side. It must be granted then that the heart may thus slowly sink, first to one side and then to another, as the position of the body is changed, the lungs adapting themselves to these changes. In the unopened chest any rebound from the position assumed by the heart during diastole will not only be against gravity (as in the opened chest) but it will encounter the mechanical difficulty of compressing the lung above it and stretching the lung below it. We should expect therefore from *a priori* reasoning that the movements of the heart from place to place within the chest cavity during each cardiac cycle to be less than occurs in the opened chest.

In order experimentally to test this point, needles were placed through the anterior chest wall into the heart near its apex. If the needles pierced the middle of the heart no lateral movement to the right was observed; if the needle pierced the heart to the left the needle (not the straw) moved to the right; if the heart was pierced to the right the needle moved to the left. This shows that while the heart contracts from side to side there is no shifting of the organ as a whole to the right to any appreciable extent.

As already mentioned light and perfectly balanced needles pushed laterally into the heart through the chest wall show that the front of the heart pushes to the sternum during diastole and moves away during systole when the animal is upon its back.

Confirmatory evidence upon these questions may be obtained by the help of the frozen sections made through the thoraxes of the dogs killed with heart in systole and diastole.

In examining the position relative to the anterior chest wall in the sections and photographs, some slight difference was observed. In the dilated heart, the anterior surface of the organ towards its apex was closely applied to the chest wall, the visceral pericardium lying in contact with the parietal layer. The heart in systole on the other hand did not show such intimate apposition.

An attempt was made to obtain some idea of the degree of movement of the heart to the right and to the left respectively. For this purpose, measurement of the area of the sections was had recourse to, and this was done in a simple manner by means of squares. A glass

slide ruled into squares was placed across the photograph, and the number of squares on each side of the middle line to which the area of the heart extended was carefully counted and the whole expressed in comparative numbers. Then the area to the right of the middle line was regarded as 1, there being less area on that side, and the left was expressed as 1 plus a fraction. From a series of measurements we obtained the following:—

Heart (Dog). Area at different levels.

(*a*) Dilated.

	Right	Left
Base	1	1·39
Middle	1	1·7

Apex 1·42 cms. to left.

(*b*) Contracted.

	Right	Left
Base	1	1·2
Middle	1	1·7

Apex 1·68 cms. to left.

From a consideration of the above figures, a close correspondence between the two sets of sections is observed; indeed those obtained from the middle of the heart show it to be absolute. This is clearly an indication that the lateral movement of the whole organ in space is very slight; the sectional area diminishes of course during contraction but it possesses practically the same relation to the middle line of the body as during extreme relaxation.

An estimation of the position of the inter-ventricular septum in the state of contraction and relaxation respectively was made by measuring the angle it formed with the mesial plane of the body. These angles were found to correspond closely, affording an indication that the movement of the septum to either side and consequently the rotation of the heart was very slight indeed and quite inappreciable. The position of the septum was more affected, by the state of distension of the right ventricle. When the right ventricle for instance was more distended than the left, the angle of the septum was greater, that is to say, the anterior edge of the septum came more to the left, showing that the distended ventricle had pushed over the septum somewhat.

In an examination of the heart in the open chest, while artificial respiration is maintained, we may see in a striking manner what we have

already insisted upon, viz. the alteration of the heart's movement under the influence of gravity. When a dog, for example, is placed symmetrically on its back and the lungs allowed to collapse, the heart falls backwards and to the left during diastole. With each systole it springs upwards and towards the right, and the twisting movement from left to right as noted by Harvey is observed. This movement may be made out more definitely by watching the line of the inter-ventricular artery and vein. This moves to the right, and it is easily seen that the twisting motion is most pronounced under these conditions. If we now inflate the lungs so that the heart is fully supported by the lungs on both sides, no rotation of the organ as a whole takes place. Around the extreme apex, the superficial muscular fibres being arranged spirally, give the appearance during contraction of a faint twisting movement, but this is confined to the surface and does not affect the heart as a whole, and this view is confirmed by the fact that there is no deviation of the inter-ventricular line during systole when the heart is fully supported.

The Heart's Impulse.

It may not unreasonably be asked what is the cause of the apex beat, if it be held that the movements of the heart within the chest cavity during the cardiac cycle are very slight, and if the front-to-back diameter of the heart diminishes during systole? We maintain that when an individual lies upon the back the heart sinks slightly under the influence of gravity, and is covered in front by the lungs, except perhaps in the region of the apex. During systole the base and middle of the heart, owing to its diminished girth, recedes still more from the front of the chest, often producing an appreciable sinking in the second and third interspaces on the left side. This may also be the case at the region of the apex, and with adherent pericardium there may be a pulling in of the chest wall sometimes observed by the physician. More frequently the apex, applied to the front of the chest during diastole, is distorted by it, reacting in systole against the distorting force (see Fig. 5 *a*) and giving the apex beat. When the body lies upon the belly the conditions are modified. The heart in virtue of its gravity sinks somewhat upon the front of the chest, and the base, middle and apex come in closer contact with the chest wall. They are pressed upon by the chest wall, and during systole the heart reacts against

the chest, giving a beat which is diffuse, and can be felt over the whole heart. Though the heart diminishes in front to back diameter, the wall of the heart remains closely applied to the chest wall—owing to the weight of the heart (see Fig. 5 *b*).

FIG. 5.

We have to express our very warm thanks to our colleague Professor Alfred Hughes. He kindly undertook to make for us the tracings, &c. and superintended our freezing operations and the cutting of our sections.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

January 11*th*, 1896.

CHARING CROSS HOSPITAL.

The effect of CO_2 upon nerve and the production of CO_2 by nerve. BY A. D. WALLER.

PRELIMINARY. Considered with reference to the current of action, isolated nerve may be recognised as existing in three states or stages, which may be roughly characterised as (1) fresh, (2) transitional, (3) stale or modified.

During the first stage, the galvanometric response to excitation consists in a large negative deflection succeeded by little or no positive after-deflection.

During the second stage, the response consists in a small negative deflection succeeded by a large positive after-deflection.

During the third stage, the response consists in a positive deflection succeeded by a positive or by a negative after-deflection.

This division into three stages is of course more or less arbitrary, the transitions from first to second and from second to third being not sudden but gradual; during the first and second stages the negative effect progressively diminishes and the positive after-effect progressively increases; late in the second stage the negative effect is vanishing, early in the third stage the positive effect is appearing, and there is thus an intermediate period in which excitation gives no visible effect followed by a positive after-effect; this period has been assigned to the second stage.

With regard to the third stage I have been led to distinguish two kinds of response according as the positive deflection has been followed by a positive or by a negative after-deflection.

The characterising features of the three stages respectively are:

of the 1st stage, a predominant negative effect. S.

of the 2nd stage, a predominant positive after-effect. N.

of the 3rd stage, a predominant positive effect. N.

The negative effect is du Bois-Reymond's "negative Schwankung," the positive after-effect is presumably Hering's "positive Nachschwankung," the positive effect (as far as I know) has not hitherto been described.

In accordance with the plan of recording previously described and demonstrated (*Proc. Phys. Soc.* Nov. 1895), *i.e.* current of injury upward or northward, negative variation downward or southward, these statements are contained in the following "Formula of Deflections."

Isolated nerve	During excitation	After excitation
1st stage	S	n
2nd stage	s or O	N
3rd stage	N	N or s

The successive stages have presented themselves most clearly and quickly in summer and autumn; in winter they have been less apparent, nerves having then often remained for more than 24 hours in the second stage s.N., occasionally (*e.g.* in January and with strong excitation) exhibiting a deflection that might have been expected to be more common, viz. a negative effect followed by a negative after-effect.

The principal object of the present communication is to exhibit individually and comparatively:

A. The effect of CO_2 upon nerve in these three states.

B. The effect of prolonged tetanisation upon nerve in these three states.

Stated *seriatim* the facts, apart from all hypothesis, are as follows:

1. The effect of a small quantity of CO_2 upon nerve in the first stage is an augmentation of the normal deflection.

2. The effect of prolonged tetanisation (5 minutes) upon nerve in the first stage is an augmentation of the normal deflection.

3. The effect of a small quantity of CO_2 upon nerve in the second stage is an augmentation of s. and a diminution of N.

An original deflection sN is replaced by a deflection Sn.

Otherwise stated the relation or quotient $\frac{\text{S or } - \text{ deflection}}{\text{N or } + \text{ after-deflection}}$ is increased.

4. The effect of prolonged tetanisation upon nerve in the second stage is an augmentation of the above defined quotient $\frac{S}{N}$.

5. The effect of CO_2 in small quantity upon nerve in the second stage, giving a response ON, is to substitute for it the response S.N.

6. The effect of prolonged tetanus upon nerve in the second stage, giving the response ON, is to substitute for it the response S.N.

Nerve in the third stage reacts in two apparently different ways to CO_2 and to tetanus, which for convenience may be designated as "clear" or "disguised." Strict parallelism of statement concerning the effects of CO_2 and of tetanus might here be misleading; the experimental results frequently diverge in the two cases, in so far that CO_2 most frequently gives a "clear" result when tetanus only gives a "disguised" result. The experimental data are accordingly more properly expressed in the following contingent statements:

7. With nerve in the third stage, giving the deflection N and the after-deflection S or N, the effect of prolonged tetanus (5 minutes) is either *a* or *b*:

(*a*) substitution of the response S.N., viz. reversal of the deflection N during excitation, being what has been designated above as the "clear" result.

In such case the regular effect of CO_2 is to produce a similar reversal. The reversal is in both cases temporary.

or (*b*) simple diminution of the original N deflection during excitation, being what has been designated above as the "disguised" result.

In such case the effect of CO_2 has been either to produce a similar diminution of N, or an actual substitution of S, generally the latter.

With regard to statements 7*a* and 7*b* relating to the two effects of tetanus, it is to be remarked that they are not contradictory, but complementary. In both cases we have a diminution of N—in (*a*) amounting to substitution of S, in (*b*) not reaching even to abolition of N.

With regard to the corresponding statements under 7*a* and 7*b* relating to the effects of CO_2 as compared with those of tetanus, that under 7*a* needs no comment, and the divergence expressed under 7*b* is perhaps not surprising, inasmuch as in stale nerve CO_2 from without

might be expected to effect more completely a change that tetanisation has only effected less completely. And in point of fact we may sometimes successively demonstrate upon a nerve in this state a diminution of N by tetanus, a diminution of N by little CO_2, and a temporary substitution of S for N by more CO_2.

We cannot from the character of the deflection of the third stage foretell with certainty what will be the character of the response, i.e. whether the N deflection will be reversed or only diminished. We may however with certainty (as far as my experience has gone) make this forecast, as soon as we have witnessed the final deflection occurring at cessation of the prolonged tetanus. This terminal deflection ω may be N or S. If it is N the next and subsequent deflections will be S diminishing, if it is S the next and subsequent deflections will be n. increasing.

8. With nerve in the three stages above described the deflections at outset of, during, and at the end of a prolonged tetanus are as follows:

	a	During	ω
1st	S	S. ward	N
2nd	s	S. ward	N
3rd	N	S. ward	N or S

Whenever the terminal deflection ω is N, the effect of tetanus comes out "clear"; whenever ω is S, the effect of tetanus comes out "disguised." And it is probable (but not yet sufficiently certified by experiment) that the direction of ω depends upon the duration of tetanus in relation with the state of nerve.

On review of any considerable number of records it will be noticed that with nerve in the first and second stages, tetanus (and CO_2) give rise to an augmented diminishing deflection, and that with nerve in the third stage tetanus (and CO_2) may give either an augmented diminishing or a diminished augmenting deflection; the diminution by tetanus in this stage simulates a fatigue effect, but is evidently of the same nature as the undisguised augmentation of S in the first and second stages.

Staircase effects. Under favourable conditions, and more especially during the second and third stages, a similar effect (augmentation of S, diminution of N) may be witnessed in progressive development in the

course of the series of brief tetani of 7·5 secs. duration at minute intervals used to excite the nerve.

The alteration taking place in the deflections from isolated nerve is very similar to the well-known staircase phenomenon occurring in contractile tissue (medusa, heart, skeletal muscle) and, considered in connection with the evidence already offered, is probably a CO_2 phenomenon. (Whether or no the phenomenon is of CO_2 production in muscular as well as in nervous tissue is for the present left an open question.)

The essential points concerning the staircase deflections in isolated nerve may be briefly expressed as follows:

9. The negative deflections of nerve in the first and second stages progressively increase at each brief tetanus (7·5 seconds), with a diminishing increment. (This increase is apparently more pronounced in the second stage than in the first.)

10. The positive deflections of nerve in the third stage progressively diminish at each brief tetanus, with a diminishing decrement.

The diminution may in certain instances be such that the positive deflection falls to zero, and passes into a gradually increasing negative deflection.

CONCLUSION. Subject to the explanatory comments in connection with particular statements, these may be still further summarised in the following table, thus bringing into clearer relief that the proposition common to all is that in all states of nerve the effect of CO_2 and the effect of prolonged tetanisation consist essentially in an augmentation of the negative (S) effects of excitation.

Stage	Type	Defl. after CO_2	Defl. after Tet.	*a*	During Tet.	*ω*
I	S	augm. S	augm. S	S	S	N
II	sN or oN	S	S	s or o	S	N
III	NN or Ns	S or n	S or n	N	S	N or S

Apart from their interest *in se* the results are presented in establishment of the conclusion that tetanised nerve evolves CO_2, and in this connection the experiments belonging to statements 1 and 2 and 7 (augmentation of a negative deflection by CO_2 and by tetanus, and reversal of deflection by CO_2 and by tetanus) may be indicated as being the most directly cogent.

Note. The oscillation period of the galvanometer magnet is in most experiments 15 secs.; in some of the earlier observations, *e.g.* in 741, it was 8·5 secs. The decrement in the first case is approximately = 3, and in the second case = 2.

The duration of excitation in all experiments was 7·5 secs. at intervals of 60 secs., with a strength of 10 units of a Berne coil (secondary 37 cm. distant from primary, 70—75 interruptions per second).

With the magnet at the period of 15 secs. and an excitation time of 7·5 secs., the value of a purely instrumental swing-back should be ·58 the original swing, and departures from such a value should be indicative of a positive or negative after-effect. With the magnet at the period 8·5 secs., and decrement = 2, it should be ·7. With the period 12 secs. and the decrement = 2·1 it should be ·69. But taking into account the duration of a tetanic state (= 7·5 secs.) we may not directly correct for the instrumental swing-back, which must be measured under the conditions of experiment with *e.g.* a pressure of 0·001 volt. lasting via the interrupter 7·5 secs. This gave for the swing-back at the periods 15, 8·5, 12 the values ·5, ·3, ·66 of the swing. These are the numbers taken into reckoning in the estimation of physiological after-effect contributive to the after-deflection, and in alterations of the $\frac{S}{N}$ quotient. Inferences are however based on none but very coarse differences, *e.g.* a swing-back equal to or greater than the original swing or the suppression of a swing-back. Such changes are obviously not concealed by instrumental oscillations. Finer changes which may have occurred have not been considered inasmuch as their measurement under the conditions of observation would have been attended by too great uncertainty; for instance, in fig. 741 no attempt is made to say whether or no there is positive after-deflection in the back-swing, and all that is inferred is that during the first stage the relation $\frac{S}{N}$ is not grossly altered by tetanus. It is obvious that no uncertainty is presented by such coarse effects as reversal of deflection, suppression of swing-back &c.

There can *e.g.* be no doubt that in 710, 762 a positive after-deflection is present, and that in both cases the quotient $\frac{S}{N}$ is greater after CO_2 and after tetanus. There may *e.g.* be doubt of the presence of a negative after-deflection in 982, 984; there can be no doubt of the presence of a positive after-deflection in 858, 859; and there can be no doubt that the after-deflection is more positive in 858, 859 than in 982, 984. The substitution of S for N by CO_2 and by tetanus in 858, 859 is obviously not open to question. The most doubtful case is that of a S after-deflection following a N deflection as in 758.

N.B. These references apply to records demonstrated at the meeting and not here reproduced.

PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

February 15*th*, 1896.

KING'S COLLEGE, LONDON.

Effects of CO_2 and of Et_2O upon the polarisation increment, upon electrotonic variations, and upon electrotonic currents. A. D. WALLER.

With regard to the mechanism of a positive variation, it is difficult to give any one sufficiently justified explanation, exclusive of a variety of alternative possibilities.

(1) Does it depend upon a predominant change at the transverse or at the longitudinal lead-off? (Probably the latter.)

Assuming it to arise at the longitudinal lead-off we have further to ask—

(2) whether it is a true positive variation of the same order as the negative variation of Du Bois-Reymond and the positive after-variation of Hering,

(3) or, whether it is a predominant anelectrotonic current aroused by the exciting induction currents. (It is little or not at all affected by the direction of these currents),

(4) or, whether during tetanisation by alternate make and break shocks, v. Fleischl's intrapolar effect in the break direction gives rise to an extra-polar effect in the same direction, viz. towards the excitation region (+ or N) when the break shock is from the led-off region. (It is little or not at all affected by the direction of induced currents),

(5) or, whether it is a negative variation of a katelectrotonic current of similar origin. (There is no evidence of the possibility of a predominant katelectrotonic current produced by induction currents alternately towards and from the led-off region of a nerve),

(6) or, whether it is a "polarisation increment" of a current in the direction of demarcation current?

Each of these questions calls for particular investigation, before we have any right to regard a positive variation as simply the converse of Du Bois-Reymond's negative variation, and this main question is complicated by the theoretical difficulty of sharply distinguishing between electrotonic and true action currents. (Practically they are distinguished by the effect of length of nerve between exciting and leading off electrodes.)

A distinction between the two kinds of effects as physical (electrotonic currents) and physiological (action currents) is not upheld by experiment, nor may we even, as far as I know, sharply distinguish between physiological and physical electrotonus, admitting (with Biedermann) kathodic and after-anodic effects to be physiological, and extra-polar polarisation effects (such as may be reproduced on core-models, Pt wire in $ZnSO_4$, or in NaCl) to be purely physical.

It is obvious that all the effects referred to are strictly speaking physical, but the additional characters by which such physical changes are recognised to be peculiar to living matter are not absent in the ordinary extra-polar electrotonic effects due to Hermann's core-polarisation.

Such polarisation (although it may be imitated on the wire model) is not in the case of normal nerve purely physical, but conditioned by the unstable equilibrium of the constituents of the living medullated fibre. As has been shown by Hermann *living* as contrasted with *killed* medullated nerve exhibits a peculiarly intense polarisation counter-effect, developing with hardly measurable rapidity, followed by a correspondingly complete and rapid depolarisation.

In harmony with this I find that the ordinary electrotonic currents (which one is perhaps tempted to regard as signs of a purely physical polarisation, such as occurs in the core-model) are subject to the temporary influence of anæsthetics, and are therefore presumably of physiological nature.

Plates 2195, 2200, 2157, exhibit a series of such currents at regular intervals, and their *temporary* modification under the influence of CO_2 and of ether.

This has been the extreme case of an apparently purely physical phenomenon demonstrated to be dependent upon a physico-chemical disposition peculiar to what we are forced to indefinitely designate as the living state.

Short of this extreme case there are several other related effects in which we shall more readily designate the essential condition of their manifestation by the indefinite expression "the living state of matter." These are:

(1) The polar action currents during kathodic and after anodic influence, independent of distance between excited and led-off points, termed by Biedermann "physiological electrotonus." These have been shown by him to be temporarily suppressed by ether, *i.e.* to be subject to true anæsthetic influence, and are now only mentioned.

(2) The negative variation of electrotonic currents—viz. a negative or S swing during brief tetanus, of a positive or N steady deflection produced by an anelectrotonic current; and a positive or N swing during brief tetanus, of a negative or S steady deflection produced by a katelectrotonic current.

These are shown to be physiological by the action of CO_2 and of Et_2O. (Plates 2181, 2185, 2209.)

(3) The positive variation of polarising currents—viz. a positive or N swing during brief tetanus, of a positive or N steady deflection produced by a weak polarising current; and a negative or S swing during brief tetanus, of a negative or S steady deflection produced by a weak polarising current.

These variations are shown to be physiological by the action of CO_2 and of ether (plates 2203, 2204, 2128).

Plate 2128 exhibits the temporary conversion of a positive or N polarisation increment into a negative effect under the influence of CO_2, viz. the temporary replacement of a polarisation increment by a polarisation decrement.

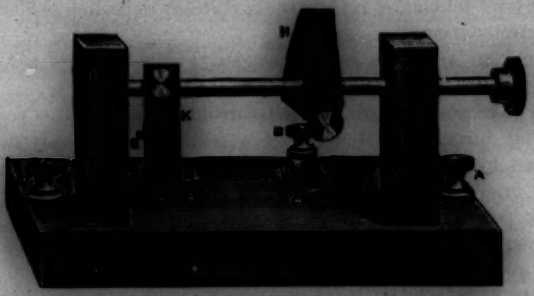
The chief conclusions to be drawn at present from these experiments are that:

The ordinary electrotonic currents of normal nerve cannot be characterised as purely physical, nor distinguished from true physiological currents by means of anæsthetics. The true negative variation (Du Bois-Reymond), the positive after-variation (Hering), the positive variation described in my last communication, the negative variation of an electrotonic current, the positive variation of a polarising current, and the electrotonic current itself are one and all subject to temporary modification by anæsthetics, and are thereby to be included in one class as physiological phenomena peculiar to the living state.

Polarising currents (·001, ·01, ·1 volt) sent through a nerve, are not appreciably altered by CO_2, Et_2O and $CHCl_3$. (Plates 2207, 2208, 2174.)

A key for cutting out either the make or break shocks produced in a secondary circuit. By T. Gregor-Brodie.

At the meeting at Charing Cross Hospital about a year ago, I showed two forms of mercury key which I had devised for the above purpose. On further experience with them, I found that the contact with the mercury and copper was not sufficiently good to completely cut out a shock if above a medium strength. I have accordingly had made a key on exactly the same principle, in which the contacts are made by firm pressure of two clean platinum surfaces. Two binding screws *A* and *B* are fixed to two tongues of metal *C* and *L*; on *C*, which is fixed, is a platinum point directed upwards to form a contact with a platinum plate



on the under surface of the tongue *L* which can be depressed. To the two binding screws *D* and *E* are fixed exactly similar tongues *F* and *M*. These form two keys which can be closed by two rotating sectors of vulcanite *H*, *K* held on a horizontal axis. They can be set at any angular interval upon this axis. Thus supposing when the key *DFE* is closed the circuit through the secondary is short-circuited, and when the key *ACB* is closed the circuit through the primary is made, then if the sectors are rotated so that the first key is closed shortly before the second the sequence of events is: First the secondary circuit is closed by the key *DFE* then, while still closed, the key *ACB* is shut and the make-shock is therefore short-circuited. On further rotation, the key *DFE* is opened and shortly afterwards *ACB* is opened and the break-shock not being short-circuited can be utilised for any purpose. By reversing the angular interval between the two vulcanite sectors we can, in a similar way, cut out the break-shocks, and only use the make-shocks.

The influence of temperature upon the extensibility of muscle. By S. W. F. Richardson and T. Gregor-Brodie.

The method we have employed is to record, by a photographic method, the variations in length of muscles stretched by different loads under a slowly rising temperature.

The muscles selected were those in which the muscle fibres are nearly parallel to one another, viz. the sartorius, and Wundt's preparation. In a few cases the gastrocnemius was also employed. In the first series the sartorius was selected because the muscle is thin and rapidly responds to any alterations in temperature of the surrounding medium. Wundt's preparation, though much thicker, was afterwards used as the two bony attachments are very strong, and stand very well the higher loads we have employed, whereas in the sartorius, with correspondingly heavy loads, the attachments continually gave way as the temperature rose. Our experiments fall into two groups; in the first the magnification is low—about 8—and is used to show the large alteration in length between 30° C. and 50° C.; in the second the magnification is high—about 50—to show the much smaller alteration occurring between 0° C. and 30° C.

With very small loads we find two shortenings superposed one upon the other. The first one starts at the usual temperature of heat-shortening—34° C.—the second at about 43° C.; the amount of shortening in each case is about the same.

With a slightly higher load, 2, 3, or 4 g. only one shortening occurs, commencing at about 34° C. and the ascent is very steep. As the load is increased the temperature at which shortening commences remains the same, the amount of shortening rapidly diminishes, and the rate with which it is carried out decreases as shown by diminishing steepness of the curve. As the load is further increased these changes become still more marked until at last a load is reached at which no contraction occurs, the curve remaining straight. For sartorius this occurred at 25 g.; for Wundt's preparation at 250 g.

With still further increase in the load an extension is produced with rising temperature, commencing at about 30° C., if the load be not too great, or at earlier temperatures when the load is very great. The curve becomes convex to the abscissa line and soon ends in rupture of the muscle, at a temperature which depends upon the amount of loading.

In the second series of experiments we have examined the small changes in length occurring at low temperatures.

With very small loads, 2, 3 or 4 g. for a sartorius, as the temperature rises shortening occurs, which gradually becomes more rapid until it ends in the main shortening beginning at about 34° C.

With higher loads, we find there is a preliminary extension commencing at about 10° to 12° C. and continuing up to the onset of heat contraction. With still heavier loads the preliminary extension begins at a slightly lower temperature.

As the load increases the amount of this extension gradually increases.

That it is not due to after-extension produced by the load is shown by the sudden alteration in the curvature at the point at which it commences.

In all cases too the load was allowed to extend the muscle for some time before the experiment was commenced, and a short tracing was also taken at constant temperature to show the rate of change of length, if any, still being produced as after-extension.

PROCEEDINGS

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UNIVERSITY COLLEGE, LONDON.

An experiment on the effect of complete removal of the parotid and submaxillary glands. By E. A. SCHÄFER, F.R.S., and B. MOORE.

We have completely removed in a dog both parotids and both submaxillary glands, together with as much as possible of the sublinguals, with the object of determining whether any of these glands possesses an intrinsic internal secretion, necessary to the life of the animal or having any important action on any of the metabolic processes.

The results of the experiment point to the conclusion that these glands resemble the spleen in not having an intrinsic secretion exercising any vitally important function. If the salivary glands do furnish an internal secretion it must be one common in character and function with that of some other organ, or at least one which can with ease be vicariously supplied by another organ.

Four operations were performed in removing the glands; before and after each operation the total nitrogen in the ingesta and egesta was determined, and the urine tested for sugar and albumen. In no case was there any disturbance found in the nitrogenous metabolism, nor was either sugar or albumen found in the urine. Carbohydrate digestion seemed to be quite unimpaired after all the salivary glands

had been removed, the dog thriving well on a diet consisting chiefly of bread and milk.

The following are the details of the experiment:—

		Wt. of dog.	Wt. of gland.
Nov. 22, 1894.	Right Parotid removed.	5·6 kilogs.	1·9 gms.
Jan. 10, 1895.	Left Parotid removed.	5·28 „	2·6 „
June 24, 1895.	Right Submaxillary and greater part of Sublingual removed.	5·95 „	2·95 „
July 22, 1895.	Left „ „	4·5 „	2·99 „
Jan. 15, 1896.	Dog killed by chloroform.	6·0 „	

About a month after the last operation, when the dog had recovered its normal weight and was in good health, it was chloroformed and a sufficient dose of pilocarpine nitrate administered to cause a copious flow from the nose and eyes and to induce severe diarrhœa. This treatment caused no flow from the mouth, but a very small quantity of exceedingly thick viscid mucus formed which was scraped off with a spatula and washed off with distilled water into a beaker. Tested afterwards, this material showed a feeble digestive power on starch, giving after half-an-hour's action on a half per cent. solution of starch a very small amount of reduction of Fehling's solution not given by a control experiment on the same starch solution. The solution also required the addition of more iodine before striking a permanent blue. It must be remembered that the quantity of mucous fluid so tested was excessively small, and appeared to be formed, as far as could be observed, by the mucous glands, and not poured out by any vestiges of the sublinguals that might have been left after the operations. In a post-mortem examination no traces of any salivary glands could be detected, either parotid, submaxillary or sublingual. There was no apparent enlargement of lymphatic glands or pancreas, nor other abnormality discoverable.

Conclusion.

This experiment, so far as it goes, shows that the salivary glands do not exert any great metabolic influence apart from their external secretion as does the pancreas.

A new apparatus for estimating hæmoglobin. By GEORGE OLIVER, M.D.

The colorometric principle is followed. The distinctive features of the apparatus are the following:—

1. The employment of double in the place of single transmission of light.

2. The solution of the blood and the standard colours rest on a pure white background of precipitated lime sulphate, which forms a most delicate reflecting surface.

3. The standard is split up into a series of definite grades, each representing a 10 per cent. gradation of the scale; and the intervening units are provided for by a series of riders.

4. Observation is made in camera through a tube mounted on a stand and specially constructed for the purpose.

The following are the parts of the apparatus: (1) an automatic blood-measurer; (2) a mixing pipette; (3) the blood-cell and cover-glass; (4) the sets of standard gradations; (5) the riders; (6) the camera tube; and (7) the pricker.

(1) *The automatic blood-measurer* has a capacity of 5 cb. mm., and fills readily by capillary attraction. It differs from the fragile capillary measures hitherto used in being made of stout glass, and therefore serviceable; in being larger and more maniputable; and in having the end well polished, so that all traces of blood can be entirely removed from it by the finger. The bore is dried out before an observation by passing a needle threaded with darning cotton through it. The handle is used for stirring together the blood and water in the blood-cell.

(2) *The mixing pipette* is provided with a rubber nozzle which fits over the blood-measurer and ensures the complete rinsing out of the blood with the first few drops of water.

(3) *The blood-cell* is of more than sufficient capacity to ensure the complete liberation of the hæmoglobin. It yields a blood solution of rather less than 1 per cent. when first filled level with the rim.

(4) *The standard gradations* are arranged as circular discs in two slabs—six in each; and they represent the divisions of 10 per cent. of the scale from 10° to 120°.[1]

The gradations have been carefully matched separately by the

[1] The quantitative equivalence of the scale in hæmoglobin is now being determined.

colour-standard glasses introduced by Mr Lovibond in his admirable system of colour measurement, and are set up accordingly. These separate estimations were found to be necessary, because the 'specific colour curve' of the blood, observed in progressive percentage dilutions, must be followed; and this is a fact which has not been before recognized. The colour composition of the gradations is so remarkably different in day-light and in candle-light that a separate set of standards is required for each.

The advantages of this apparatus are mainly in the direction that it renders observation rapid and more definite and accurate than has hitherto been attainable.

A new method of estimating the percentage of the blood-corpuscles. By GEORGE OLIVER, M.D.

When the long diameter of a flattened tube, filled with water, is directed towards a candle-flame a transverse line of bright illumination is seen, consisting of closely packed minute images of the flame produced by the longitudinal fibrillation of the glass. When a certain measure of blood (*e.g.* as in this observation 10 cb.mm.) is washed into such a tube by means of Hayem's or other fixing fluid the illuminated line is completely shut out of view, until at a certain degree of dilution it can just be detected as a delicate streak of light across the tube. Repeated observation has shown that the development of this horizontal line by dilution with a fixing fluid is a very sensitive indicator of the percentage of the blood corpuscles; for example, differences of from $\frac{1}{2}$ to 1 per cent. being readable in a blood of 100 per cent. It has been found that the proteids of the plasma do not constitute a source of error with Hayem's solution, which is preferred for this observation. The tube has been graduated from human blood. The observation is made at the distance of 10 feet from the candle-flame.

Some Improvements in the Rocking Microtome (with demonstration). By HERBERT E. DURHAM.

There are some defects in the ordinary form of rocking microtome; but the following important ones are readily overcome.

I. A very limited portion of the available razor edge can be used. This I have remedied by having the entire razor holder removed, and the front part of the bed of the microtome planed down

flat. A movable razor holder is substituted: it can be moved about laterally, backwards and forwards, or placed at any angle should this be desired. When put in the desired position it is clamped by a fixing bolt.

By means of this the whole of the available razor edge may be utilized; also a certain amount of orientation may be obtained.

II. I have found that with my instrument the block of paraffin was not clear of the edge of the razor on the upward stroke before the throw forward commenced. This took place even with moderate sized blocks, and was brought to my notice practically during the employment of the instrument. It was due to the fact that the stop for the pawl was in a faulty position (this stop varies in position very considerably in the different rocking microtomes I have examined). The correct principle to use is to adjust the movable sector so that the paraffin block is entirely above the razor edge before the pawl strikes the milled wheel; the thickness of section being adjusted by means of a movable stop. This is easily effected by replacing the fixed stop by a fixed block through which a strong well-fitted screw passes horizontally; one end of the screw forms the movable stop, the other has a handle in order to facilitate turning it.

Besides the advantage of the correctness of the principle, the convenience and the delicacy of the adjustment are found to be far greater than is the case when the sector itself has to be moved.

There are two further points which may be noted in the instrument I use. First with regard to the object holder; this consists of a steel rod, which passes into a hole bored out in the rocking lever, at the end of which a thin flange of brass is soldered. The surface of the brass flange has a few cuts upon it whereby the paraffin holds firmly.

The advantage which this form of holder presents is that objects about 2 inches long can be mounted if desired; the maximum length which the ordinary form is capable of taking is about $\frac{3}{4}$—$\frac{7}{8}$ inch.

For orientating I use a simple ball and socket arrangement; consisting of a clamp, each of whose jaws has a spherical depression, and they are approximated by a single strong clamping screw: this is mounted on a steel rod which fits the bore of the rocking lever. The brass flange for fixing the paraffin has a stem which ends in a ball; the ball fits the depressions in the clamp, and thus the paraffin block may be fixed at any desired angle or position. Naturally the spherical joint allows of both angular and rotatory adjustment.

Secondly, it is often advantageous to fix the rocking lever at any given point. This is effected by means of a sliding collar running along the rocking arm and provided with a fixing screw. The actuating cat-gut string is attached to this collar, and by sliding the collar the rocking lever may either be fixed at a given point, or the length of throw regulated. As the collar is fixed by means of a screw there is no chance of its sliding from its position.

Certain of the above arrangements have been made for me by Mr J. A. Prestwich[1]: the nicety and finish of construction being most admirable and leaving nothing to be desired.

[1] Mr Prestwich, of 744, High Road, Tottenham, London, N., tells me that he will be willing to alter any existing rocking microtome according to the above description: he is in possession of the details of measurement etc., which seemed hardly worth while going into in the above note.

END OF VOLUME